

VIRULENCE GENES, PROTEINS, AND THEIR USE

FIELD OF THE INVENTION

This invention relates to virulence genes and proteins, and their use. More particularly, it relates to genes and proteins/peptides obtained from gram-negative bacteria,
5 and their use in therapy and in screening for drugs.

BACKGROUND OF THE INVENTION

According to health care experts, infectious diseases caused by microbes are responsible for more deaths worldwide than any other single cause. The current estimate of the annual cost of medical care for treating infectious diseases in the United States alone is
10 about \$120 billion. While antibiotic treatment is effective for many microbial infections, antibiotic resistance among pathogenic bacteria is a growing health concern. Indeed, the American Medical Association has concluded that, "the global increase in resistance to antimicrobial drugs, including the emergence of bacterial strains that are resistant to all available antibacterial agents, has created a public health problem of potentially crisis
15 proportions."

Pseudomonas and *Klebsiella* are two genres of gram-negative bacteria that pose a significant health risk to infected host organisms, in part, due to their resistance to many antibiotics. These bacteria are noted for causing life-threatening infections, particularly in the lung. Cancer and burn patients also commonly suffer serious *Pseudomonas* infections, as do
20 certain other individuals with immune system deficiencies. While *Klebsiella* sp. is responsible for many types of infections, outside of a medical setting, the most common infection caused by *Klebsiella* bacteria is pneumonia.

There is a need in the art for new antimicrobial therapeutic strategies.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery of 46 genes, when mutated lower the virulence of a gram-negative bacterium, and can be used in new antimicrobial therapeutic strategies. The invention provides attenuated bacterial mutants that are derived from pathogenic strains. These attenuated bacterial stains have a mutation in a VIRX gene identified herein as VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and VIR46; and show reduced inhibition of *Dictyostelium* amoeba growth when compared to the growth observed in the presence of an isogenic bacterial strain. The term, "pathogenic," as used herein, is defined as an agent's ability to cause disease, damage or harm to a host organism. The term, "attenuated," as used herein, means an organism made less virulent relative to an isogenic pathogenic organism. The term, "mutant," as used herein, an organism carrying a specific mutation of a gene that is expressed in the organism's phenotype. A mutation may be insertional inactivation or deletion of a gene. It is preferred that the mutation be an insertional inactivation of a gene.

The invention also provides attenuated bacterial mutants that are derived from pathogenic gram-negative bacterial strains. These attenuated gram-negative bacterial strains have a mutation in a VIRX gene identified herein as VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and VIR46; and show reduced inhibition of *Dictyostelium* amoeba growth when compared to the growth observed in the presence of an isogenic bacterial strain. A mutation may be insertional inactivation or deletion of a gene. It is preferred that the mutation be an insertional inactivation of a gene. It is also preferred that the attenuated gram-negative bacterial mutant be derived from a *Pseudomonas* or *Klebsiella* spp. It is more preferred that the attenuated gram-negative bacterial mutant is a strain of *P. aeruginosa* or *K. pneumoniae*.

The invention additionally provides for a VIRX gene that may be part of an operon. The term, "operon," as used herein, is a unit of bacterial gene expression and regulation

comprising several genes, usually with complementary functions. Insertion in a gene in an operon typically interferes with the function of this gene and of other genes located downstream or upstream in the operon. The function attributed to a gene refers to its function and/or that of any gene located downstream or upstream in the same operon. Accordingly, the invention also provides for a bacterial strain comprising an operon encoding a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR44, VIR45, and VIR46, wherein the bacterial strain includes a mutation that reduces expression of the VIRX gene relative to an isogenic bacterial strain lacking the mutation. In one embodiment, the the mutation reduces inhibition of *Dictyostelium* amoeba growth when compared to the growth of *Dictyostelium* amoeba in the presence of an isogenic bacterial strain lacking the mutation.

The invention provides for one or more of the following attenuated *Pseudomonas* mutant strains: MUT1; MUT2; MUT3; MUT4; MUT5; MUT6; MUT7; MUT8; MUT9; MUT10; MUT11; MUT12; MUT13; MUT14; MUT15; MUT16; MUT17; MUT18; and MUT19. The invention also provides for one or more of the following attenuated *Klebsiella* mutant strains: MUT20; MUT21; MUT22; MUT23; MUT24; MUT25; MUT26; MUT27; MUT28; MUT29; MUT30; MUT31; MUT32; MUT33; MUT34; MUT35; MUT36; MUT37; MUT38; MUT39; MUT40; MUT41; MUT42; MUT43; MUT44; MUT45; and MUT46.

The invention additionally provides a method for identifying an antimicrobial drug, wherein a candidate composition is contacted with at least one polypeptide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45 and VIR46. The biological activity of polypeptide in the presence of the candidate composition is compared with the biological activity of the polypeptide in the absence of the candidate composition. Alteration of the biological activity of the polypeptide indicates that the candidate composition is an antimicrobial drug. In some embodiments, the candidate composition contains at least two molecules. The candidate

composition can contain at least one molecule less than about 500 Daltons or at least one molecule greater than about 500 Daltons. The candidate composition can be, *e.g.*, an immunoglobulin, polysaccharide, lipid, nucleic acid, or combination thereof.

The invention additionally provides a method for identifying an antimicrobial drug, wherein a candidate composition is contacted with at least one polynucleotide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and VIR46. The expression of the polynucleotide in the presence of the candidate composition is compared with the expression of the polynucleotide in the absence of the candidate composition. Alteration of the expression of the polynucleotide indicates that the candidate composition is an antimicrobial drug. In some embodiments, the candidate composition contains at least two molecules. The candidate composition can contain at least one molecule less than about 500 Daltons or at least one molecule greater than about 500 Daltons. The candidate composition can be a polypeptide, polysaccharide, lipid, nucleic acid, *e.g.*, ribonucleic acid, or combination thereof. In a preferred embodiment, the ribonucleic acid of the candidate composition is a small interfering ribonucleic acid.

The invention additionally provides a method for determining the degree of virulence of a pathogen present in a subject, comprising:

(a) measuring the level of expression of at least one polypeptide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR43, VIR44, VIR45, and VIR46, in a sample from the first subject; and

(b) comparing the amount of the polypeptide in the sample of step (a) to the amount of the polypeptide present in a control sample from a second subject known not to have the presence of the pathogen, wherein an alteration in the

expression level of the polypeptide in the first subject as compared to the control sample indicates the degree of virulence of the pathogen.

In a preferred embodiment, the subject is a mammal. It is more preferred that the subject is a human.

5 The invention also provides a method for determining the degree of virulence of a pathogen present in a subject, comprising:

(a) measuring the level of expression of at least one polynucleotide encoded by a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, 10 VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR44, VIR45, and VIR46, in a sample from the first subject; and

(b) comparing the amount of the polynucleotide in the sample of step (a) to the amount of the polynucleotide present in a control sample from a second subject known not to 15 have the presence of the pathogen, wherein an alteration in the expression level of the polypeptide in the first subject as compared to the control sample indicates the degree of virulence of the pathogen.

In a preferred embodiment, the subject is a mammal. It is more preferred that the subject is a human.

20 The invention additionally provides attenuated bacterial strains that can be used as vaccines and as vectors for foreign antigens and for foreign DNA. These attenuated bacterial strains are useful for the preparation of vaccines effective against diseases associated with the corresponding bacterial strains. In a preferred embodiment, the attenuated bacterial strains are derived from *Pseudomonas* or *Klebsiella* spp.

25 The invention additionally provides attenuated bacterial strains that can be used as vectors for foreign genes cloned from other pathogens that will be expressed into proteins, and will raise protective immune responses against the pathogens from which they are derived. In a preferred embodiment, the attenuated bacterial strains used as the vectors are derived from *Pseudomonas* or *Klebsiella* spp.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, on the discovery of 46 genes when mutated lower the virulence of a gram-negative bacterium. Nineteen of these virulence genes were identified in *P. aeruginosa* PT894, while the remaining 27 genes were derived from mutagenesis of *Klebsiella*. These bacterial mutants have attenuated virulence relative to isogenic bacterial strains and are designated "MUTX." Provided herein are virulence genes affected in each novel, attenuated MUTX strain, as well as the nucleotides and polypeptides encoded thereby. The sequences encoded by the affected genes are collectively referred to as "VIRX nucleic acids" or "VIRX polynucleotides" and the corresponding encoded polypeptides are referred to as "VIRX polypeptides" or "VIRX proteins." Unless indicated otherwise, "VIRX" is meant to refer to any of the novel sequences disclosed herein.

The peptides and genes of the invention are useful for the preparation of therapeutic agents to treat infection because they attenuate the virulence of the wild-type pathogen. Therapy can be preventative or therapeutic. A subject receiving therapy can be, *e.g.*, a human, a non-human primate (such as an ape, gorilla, or chimpanzee), cow, horse, pig, sheep, dog, cat, or rodent (including mouse or rat).

I. IDENTIFICATION OF *PSEUDOMONAS* AND *KLEBSIELLA* GENES ENCODING VIRULENCE FACTORS

Genes encoding virulence factors (*e.g.*, pathogens or toxins) to a host organism were

identified by comparing the growth of *Dictyostelium discoideum*, in the presence and absence of test mutants of *Pseudomonas* and *Klebsiella* with an identifiable genetic alteration as detailed in International Application PCT/IB02/03277, filed June 7, 2002. *Dictyostelium* amoebae feed phagocytically upon bacteria such as *K. pneumoniae*. When *Dictyostelium* cells are plated with *K. pneumoniae* bacteria, each amoeba creates a plaque in the bacterial lawn in the region where bacteria have been phagocytosed. Addition of pathogenic bacteria, e.g., *P. aeruginosa* strain PT894 to the lawn of *K. pneumoniae* bacteria, inhibits the growth of the amoebae.

Pseudomonas test mutants were made by transposon insertion according to known methods in the art and tested for virulence in a *Dictyostelium* growth assay (see, PCT/IB02/03277, filed June 7, 2002). *Klebsiella* mutants were also made by transposon insertion according to known methods in the art and tested for virulence in a *Dictyostelium* growth assay (see, PCT/IB02/03277, filed June 7, 2002) using the *PHG1a* mutant *Dictyostelium* strain (Cornillon *et al.*, J. Biol. Chem., 275(44): 34287-92, 2000), a strain which was found to be particularly sensitive to virulent bacteria. Specifically, the *Klebsiella* mutants were obtained by standard bacteria electroporation technique using the plasposon pNKBOR (Genbank accession number: AF310136) and selected on solid LB medium containing 50 µg/ml kanamycin (Rossignol *et al.*, Res. Microbiol., 152(5): 481-5, 2001). Other mutagenesis methods known in the art, e.g., ultraviolet radiation exposure, treatment with intercalating agent or transducing phage, may also be used to generate mutants. Mutations yielding reduced virulence were identified where the growth of the *Dictyostelium* test host organism exposed to the mutant pathogen was greater than the *Dictyostelium* test host organism exposed to wild-type pathogen. Specific genetic mutations in pathogens displaying reduced virulence were subsequently identified and characterized by techniques well known in the art. Identification of specific gene mutations in *Klebsiella* mutants was performed by plasmid rescue and cloning of the genomic DNA at the insertion site mutant using the BglII or ApaI restriction enzyme according to (Rossignol *et al.*, Res. Microbiol., 152(5): 481-5, 2001). Identification of specific gene mutations in *Pseudomonas* mutants was performed by subcloning the transposon and surrounding bacteria genomic DNA into an acceptor plamid. DNA sequencing was performed on amplified rescued plasmids, in order to identify the insertion site of the transposon. Rat mortality assays such as that described by Join-Lambert *et al.*, Antimicrob. Agents Chemother., 45(2): 571-6, 2001, can be used to

corroborate attenuated virulence activity in a mammalian host.

The 19 *Pseudomonas* attenuated MUTX organisms harboring the VIRX genes are summarized below in Table 1.

Table 1

STRAIN	AFFECTED VIRULENCE GENE(S)	REFERENCE
MUT1	anthranilate phosphoribosyltransferase (trpD; PA0650)	Essar <i>et al.</i> , J. Bacteriol., 172:853-66, 1990; Essar <i>et al.</i> , J. Bacteriol., 172:867-83, 1990.
MUT2	ATP sulfurylase small subunit (CysD; PA4443)	Leyh <i>et al.</i> , J. Biol. Chem., 263:2409-16, 1988; Hummerjohann <i>et al.</i> , Microbiology, 144 (Pt 5):1375-86, 1998
MUT3	CysQ (PA5175)	Peng and Verma, J. Biol. Chem., 270:29105-10, 1995; Neuwald <i>et al.</i> , J. Bacteriol., 174:415-25, 1992.
MUT4	D-amino acid dehydrogenase, small subunit (dadA; PA5304)	Lobacka <i>et al.</i> , J. Bacteriol., 176:1500-10, 1994.
MUT5	imidazoleglycerol-phosphate synthase, cyclase subunit (hisF1; PA5140)	Fani <i>et al.</i> , Mol. Gen. Genet., 216:224-9, 1989; Fani <i>et al.</i> , Mol. Gen. Genet., 216:224-9, 1989.
MUT6	N-acetyl- γ -glutamyl-phosphate reductase (ArgC; PA0662)	Smith <i>et al.</i> , Gene, 49:53-60, 1986.
MUT7	Dihydrolipoamide acetyltransferase (AceF; pyruvate dehydrogenase complex component E2; PA5016)	Rae <i>et al.</i> , J. Bacteriol., 179:3561-71, 1997.
MUT8	NADH dehydrogenase I chain H (nuoH; PA2643)	Weidner <i>et al.</i> , J. Mol. Biol., 5:233:109-22, 1993; Weidner <i>et al.</i> , J. Mol. Biol., 233:109-22, 1993.
MUT9	pyoverdine synthetase D (PvdD; PA2399)	Rombel <i>et al.</i> , Mol. Gen. Genet., 246:519-28, 1995; Merriman <i>et al.</i> , J. Bacteriol., 177:252-8, 1995.
MUT10	RND multidrug efflux transporter MexD (mexD; PA4598)	Poole <i>et al.</i> , Mol. Microbiol., 21:713-24, 1996; Poole <i>et al.</i> , Mol. Microbiol., 21:713-24, 1996.
MUT11	PA3721	Stover <i>et al.</i> , Nature, 406:959-964, 2000.
MUT12	PA0596	Tan <i>et al.</i> , Proc. Natl. Acad. Sci. USA, 96:2408-13, 1999.
MUT13	PA5265	Stover <i>et al.</i> , Nature, 406: 959-964, 2000.

MUT14	pyochelin biosynthetic protein pchC (PA4229)	Serino <i>et al.</i> , Mol. Gen. Genet., 249: 217-28, 1995; Serino <i>et al.</i> , J. Bactiol., 179:248-57, 1997
MUT15	dihydroaeruginosic acid synthetase (pchE; PA4226)	Reimmann <i>et al.</i> , Microbiology, 144: 3135-48, 1998.
MUT16	Pyochelin synthetase (pchF; PA4225)	Reimmann <i>et al.</i> , Microbiology, 144: 3135-48, 1998.
MUT17	ATP-binding component of the ABC transporter (pchH; PA4223)	Featherston <i>et al.</i> , Mol. Microbiol., 32(2):289-99, 1999; Reimmann <i>et al.</i> , J. Bacteriol., 183:813-20, 2001.
MUT18	ATP-binding component of the ABC transporter (pchI; PA4222)	Reimmann <i>et al.</i> , J. Bacteriol., 183:813-20, 2001.
MUT19	putative O-antigen biosynthesis gene cluster	Rocchetta <i>et al.</i> , Microbiol. Mol. Biol. Rev. 63:523-53, 1999.

The 27 *Klebsiella* attenuated MUTX organisms harboring the VIRX genes disclosed in the present invention and assigned a new role in virulence are summarized below in Table 2.

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Table 2

STRAIN	AFFECTED VIRULENCE GENE(S)
MUT20	hypothetical transcriptional regulator in met G-dld intergenic region
MUT21	β -cystathionase
MUT22	ribosome binding factor A
MUT23	aspartokinase/homoserine dehydrogenase
MUT24	cystathionine γ -synthase
MUT25	Phosphoribosylformylglycinamide synthase

MUT26	homoserine transsuccinylase
MUT27	3'-phosphoadenosine 5'-phosphosulfate reductase
MUT28	Sfi protein
MUT29	transcriptional activator protein LysR
MUT30	TrpD
MUT31	N-acetylglucosamine-6-phosphate deacetylase
MUT32	WaaQ
MUT33	2-Isopropylmalate synthase
MUT34	histidinol dehydrogenase
MUT35	UDP-galactopyranose mutase
MUT36	O-antigen export system permease protein rfba
MUT37	uridyltransferase
MUT38	pyridoxine phosphate biosynthetic protein PdxJ-PdxA
MUT39	triose phosphate isomerase
MUT40	aldehyde dehydrogenase
MUT41	galactosyl transferase
MUT42	siroheme synthetase
MUT43	7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase
MUT44	glucose-6-phosphate isomerase
MUT45	DNA methylase
MUT46	putative inner membrane protein

II. ATTENUATED BACTERIAL MUTANTS

A. Attenuated *Pseudomonas aeruginosa* Mutants

MUT1

A *Pseudomonas* bacterial mutant (MUT1) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding anthranilate phosphoribosyltransferase (PA0650). This gene encodes the VIR1 nucleic acid (SEQ ID NO:1) shown in Table 3A.

Table 3A. VIR1 Nucleotide Sequence (SEQ ID NO:1)

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ATGGATATCAAGGGAGCCCTCAATCGCATCGTCAACCAGCTCGACCTGACCACCGAGGAAATGCAGG
CGGTCATGCGCCAGATCATGACCGGGCAGTGCACCGACGCGCAGATCGGCGCCTTCCTGATGGGCAT
GCGGATGAAGAGCGAAACCATCGACGAGATCGTCGGCGCGGTGGCGGTGATGCGCGAATGGCCGAC
GGCGTGCAAGTTCCTACGCTGAAGCATGTGGTTCGACGTGGTCGGCACCGGCGGCGATGGCGCGAACA
TCTTCAACGTGTCTCGGCGGCGTCTTCGTGGTTCGCGCCGCTGGCGGCAAGGTGCGCAAACACGG
TAACCGCGCGGTCTCCGGCAAGAGCGGCGAGCGCCGACTTGCCTGGAAGCCGCGGCGCATCTACCTGGAG
CTGACCTCCGAACAGGTGGCGCGTTGCATCGACACCGTTCGGCGTTCGGGTTCATGTTGCGCCAGGTCC
ACCACAAGGCGATGAAGTACGCGCGCGGTCCGCGCGGAGCTGGGCTTCGGGACTCTGTTCAACAT
GCTTGGCCCACTGACCAACCCGGCGGGAGTCAGGCACAGGTGGTTCGGGTTCACCCAGGAACTG
TGCAAGCCGCTGGCTGAAGTGCCTCAAGCGTCTCGGCAGCGAGCATGTGCTGGTGGTGCATTTCGCGCG
ACGGGCTGGACGAGTTCAGTCTGGCGCGGCGGACCCACATTGCCGAGTTGAAGGACGGCGAGGTACG
CGAGTACGAAGTGCCTCCGAGGACTTCGGGATCAAGAGCCAGACCTGATGGGGCTGGAGGTTCGAC
AGTCCGCGAGGCTTCGCTGGAACATGATCCGCGACGCTTTGGGGCGGCGCAAGACCGAGGCTGGGCAGA
AGGCCGCGGAGCTGATCGTGAATGCCGCGCGGCGACGTGTACGCTGCCGATCTGGCGACACGCT
GCACGAGGGCATTTCAACTGGCCACGATGCCCTGCACACCGGGCTGGCACGGGAGAAGATGGACGAA
CTGGTGGCCTTACCGCGCTTTACAGAGAGGAGAACGCACAGTGA
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The VIR1 protein (SEQ ID NO:2) encoded by SEQ ID NO:1 is presented using the one-letter amino acid code in Table 3B.

Table 3B. Encoded VIR1 protein sequence (SEQ ID NO:2)

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MDIKGALNRIVNQLDLTTEEMQAVMRQIMTGQCTDAQIGAFLMGMRMKSETIDEIVGAVAVMREL
ADGVQLPTLKHVVDVVGTTGGDGANIFNVSSAASFVVAAGGKVAKHGNRAVSGKSGSADLLEAAG
IYLELTSEQVARCIDTVGVGFMAQVHHKAMKYAAGPRRELGLRTLNFNMLGPLTNPAGVRHQVVG
VFTQELCKPLAEVLKRLGSEHVLVHRSRDLDEFSLAAATHIAELKDGEVREYEVRPEDFGIKSQ
TLMGLEVDSPQASLELIRDALGRRKTEAGQKAAELIVMAGPALYAADLATSLHEGIQLAHDALH
TGLAREKMDDELVAFTAVYREENAQ
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The role of VIR1 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT2

A *Pseudomonas* bacterial mutant (MUT2) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding the ATP sulfurylase small subunit (CysD; PA4443). This gene encodes the VIR2 nucleic acid (SEQ ID NO:3) shown in Table 4A.

Table 4A. VIR2 Nucleotide Sequence (SEQ ID NO:3)

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ATGGTCGACAACTGACGCACCTGAAACAGCTGGAGGCGGAAAGCATCCACATCATCCGCGAGGTGG
CCGCCGAGTTCGATAACCCGGTGATGCTGTACTCGATCGGCAAGGATTCCGCGGTCATGCTGCACCT
GGCCCGCAAGGCCTTCTTCCCCGGCAAGCTGCCCTTCCCGGTGATGCACGTGGACACCCGCTGGAAA
TTCCAGGAGATGTACAGGTTCCGTGATCGGATGGTTCGAGGAAATGGGCCTGGATCTGATCACCCACG
TCAACCCGGACGGCGTCGCCCCAGGGCATCAACCCGTTACCCACGGCAGCGCCAAGCACACCGACGT
GATGAAGACCGAGGGACTCAAGCAGGCCCTGGACAAGTACGGTTTCGACGCTGCCTTCGGCGGTGCG
CGCCGCGACGAGGAGAAGTCGCGGGCCAAAGGAACGGGTCTATTCTGTTCCGCGACAGCAAGCACCGCT
GGGACCCGAAGAACCAGCGTCCCGAGCTGTGGAACATCTACAACGGCAAGGTGAAGAAGGGCGAGTC
GATCCGCGTCTTCCCGCTGTCCAACCTGGACCGAGCTGGACATCTGGCAATACATCTACCTGGAAGGC
ATCCCGATCGTCCCGCTGTACTTCGCCGCCGAGCGCGAGGTCATCGAGAAGAATGGCACATTGATCA
TGATCGACGACGAGCGCATCCTCGAGCATCTCTTGACGAAGAGAAAGCCGCATCGAGAAGCGCAT
GGTGCCTTCCGTACCCCTCGGCTGCTACCCGCTCACCGGCGCGGTGAGTCCAGCGCCACCACGCTG
CCGGAATCATCCAGGAAATGCTCCTGACGCGTACTTCCGAACGCCAGGGCCGGGTGATCGACCATG
ACCAGGCCGGTTTCGATGGAAGAAAAGAACGTCAGGGCTATTTCTGA

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The VIR2 protein (SEQ ID NO:4) encoded by SEQ ID NO:3 is presented using the one-letter amino acid code in Table 4B.

Table 4B. Encoded VIR2 protein sequence (SEQ ID NO:4)

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MVDKLTHLKQLEAESIHIREVAAEFDPNPVMLYSIGKDSAVMLHLARKAFFPGKLPFPVMHVDTR
WKPFQEMYRFRDRMVEEMGLDLITHVNPDPGVAQGINPFTHGSAKHTDVMKTEGLKQALDKYGFDA
FGGARRDEEKSRAKERVYSFRDSKHRWDPKNQRPPELWNIYNGKVKGESIRVFPPLSNWTELDI
WQYIYLEGIPVPLYPFAAEREVIEKNGTLMIDDERILEHLSDEEKARIEKRMVRFRTLGCPYPLTGA
VESSATTLPEIIQEMLLTRTSEKQGRVIDHDQAGSMEEKKRQGYF

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The role of VIR2 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT3

A *Pseudomonas* bacterial mutant (MUT3) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated

microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding CysQ (PA5175). This gene encodes the VIR3 nucleic acid (SEQ ID NO:5) shown in Table 5A.

5

Table 5A. VIR3 Nucleotide Sequence (SEQ ID NO:5)
ATGAGGCCGGTGCCTTGGGGCGAATTGGTGGCGCTGGTGCGGCGCGCCGGCGAGGCGATCCTGCCGC ACTGGCGCGCCGACGTGGTGGTGGCGCTCGAAGGCCGACGAATCGCCGGTGACTGCCGCCGACCTGGC CGCGCACCATATATTGGAGGCGGGATTGCGGGCGCTGGCGCCGGACATTCCGGTGCTTTCCGAAGAG GATTGCGAGATACCGCTGAGCGAGCGCGGCCACTGGCGCGCGCTGGTGGCTGGTGGACCCGCTGGACG GCACCAAGGAGTTCATCTCCGGTAGCGAGGAGTTCACCGTCAACGTGGCCCTGGTCGAGGATGGCCG GGTGCTGTTCGGCCTGGTCCGGCTGCCGGTGAGCGGCCGCTGCTACTACGGTGGCGCCGGTCTCGGT GCCTGGCGCGAGGAGGCCGATGGCCGCGCGCAACCGATCAGTGTGCGCCTGGAGCCCGAGGAGGCCT TCACCGTGGTGGCCAGCAAGCGCCATGGCAGCCCGGCCAGGAGCGCCTGCTGGATGGCTTGAGCGA GCGCTTCGGCGACCTGCGGCGAGCCAGCATCGGCAGTTCGCTGAAGTTCTGCTGCTGGCCGAGGGC GCTGCCGACTGCTATCCGCGCCTGACGCCAACCTCGCAATGGGACACGGCCGCGCCAGGGTGTGC TGAAGGCGCCGGCGGCGAGGTGCTCGACCTGCATGGTGCGCCATTACCTACGAGCCGCGCGAGGA TTACCTCAACGGCTCCTTCTGGCCCTGCCGCGCGCCGCGAGTGGCGCAGCGAGCTGATCCAAC TG GCGCGCGCGCTGCACTGA

The VIR3 protein (SEQ ID NO:6) encoded by SEQ ID NO:5 is presented using the one-letter amino acid code in Table 5B.

Table 5B. Encoded VIR3 protein sequence (SEQ ID NO:6)
MRPVPWGE LVALVRRAGEAILPHWRADVVRSKADESPVTAADLAHHILEAGLRALAPDIPVLS EEDCEIPLSERGHWRRLVDPLDGTKEFISGSEFTVNVALVEDGRVLFGLVGVPSGRCYGG AGLGAWREEADGRAQPI SVRLEPEEAFTV VASKRHGSPAQERLLDGLSERFGDLRRASIGSSLKF CLLAEGAADCYPRLTPTSQWDTAA AQVLE GAGGEVLDLHGAPFTYEPREDYLN GSFLALPRAAE WRSELIQLARALH

10

MUT4

A *Pseudomonas* bacterial mutant (MUT4) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding D-amino acid dehydrogenase, small subunit (dadA; PA5304). This gene encodes the VIR4 nucleic acid (SEQ ID NO:7) shown in Table 6A.

15

Table 6A. VIR4 Nucleotide Sequence (SEQ ID NO:7)
ATGCGAGTTCTGGTCCTTGGCAGCGGTGTCATCGGTACCGCCAGTGCGTATTACCTGGCCCGTGCCG GGTTCGAGGTGGTGGTGGTCGACCGTCAGGACGGTCCCGCGCTGGAAACAGCTTCGCCAACGCCG

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CCAGGTGTCTCCCGGCTACGCTTCGCCCTGGGCAGCCCCGGGCATTCCCCCTGAAGGCCATGAAGTGG
CTGCTGGAAAAGCACGCGCCGCTGGCCATCAAGCTCACCTCCGATCCCAGCCAGTACGCCCTGGATGC
TGCAGATGCTGCGCAACTGCACCGCCGAGCGCTACGCCGTGAACAAGGAGCGCATGGTCCGCCTGTG
CGAGTACAGCCGCGATTGCCCTCGACGAACTGCGCGCCGAGACCGGCATCGCCTACGAGGGCCGCACC
CTCGGCACCACTGTTCCGACCCAGGCGCAGCTGGACGCCGCCGGCAAGGACATCGCCCGTGC
TTCGAGCGCTCCGGCGTGCCCTACGAGGTTCTCGACCGCGACGGCATCGCCCGGTAGAGCCGGCTTT
GGCCAAGGTCGCCGACAAGCTGGTTCGGCGCCTTGCGCCTGCCCAACGACCAGACCGGCGACTGCCAG
CTGTTTACCACCCGCTGGCGGAAATGGCCAAGGGCCTGGGCGTGGAGTTCGCTTCGGCCAGAACA
TCGAGCGCCTGGACTTCGCCGGCGACCGCATCAACGGCGTGTGGTCAACGGCGAATTGCTCACCGC
CGACCACTACGTGCTGGCCCTGGGCAGCTACTCGCCGCAACTGCTCAAGCCGCTGGGTATCAAGGCT
CCGGTCTATCCGCTGAAGGGTTATTCGCTGACCGTGCCGATCACCAACCCGGAGATGGCGCCGACCT
CGACCATCCTCGACGAGACCTACAAGGTGGCGATCACCCGCTTCGACAGCGCATCCGCGTCGGCGG
CATGGCGGAAATCGCCGGCTTCGACCTGTGCTGAACCCGCGCCGCCGCGAGACCTTGAAATGATC
ACCACCGACCTCTATCCCGAGGGCGGCGATATCAGCCAGGCGACCTTCTGGACCGGCTGCGCCCGG
CGACCCCGGATGGCACCCCGATCGTCGGCGCCACCCGCTACCGCAACCTGTTCCCTCAATACCGGCCA
CGGCACCCTGGGTTGGACCATGGCCTGCGGGTTCGGGTGCTACCTGGCCGACCTGATGGCGAAGAAG
CGCCCGCAGATCAGTACCGAAGGCTGGATATTTCCCGCTACAGCAATTCCCCGGAGAACGCCAAGA
ATGCCCATCCAGCGCCAGCACACTAA

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The VIR4 protein (SEQ ID NO:8) encoded by SEQ ID NO:7 is presented using the one-letter amino acid code in Table 6B.

Table 6B. Encoded VIR4 protein sequence (SEQ ID NO:8)

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MRVLVLGSGVIGTASAYYLARAGFEVVVDQRDGPALTSFANAGQVSPGYASPWAAAPGIPLKAM
KWLLLEKHAPLAIKLTSDPSQYAWMLQMLRNCTAERYAVNKMVRLSEYSRDCLDELRAETGIAY
EGRTLGTTLQFRTOAQLDAAGKDIAVLERSGVPEVLDLDRDGIARVEPALAKVADKLVGALRLPND
QTGDCQLFTTRLAEMAKGLGVEFRGQNIERLDFAGDRINGVLVNGELLTADHYVLALGSYSPQL
LKPLGIKAPVYPLKGYSLTVPIITNPEMAPTSTILDETYKVAITRFDQIRIVGGMMAEIAAGFDLSLN
PRRRETLEMITTDLYPEGGLISQATFWTGLRPATPDGTPIVGATRYRNLFNTGHGTLGWMTMACG
SGRYLADLMAKKRPQISTEGLDISRYSNSPENAKNAHPAPAH

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The role of VIR4 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT5

A *Pseudomonas* bacterial mutant (MUT5) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding imidazoleglycerol-phosphate synthase, cyclase subunit (hisF ; PA5140). This gene encodes the VIR5 nucleic acid (SEQ ID NO:9) shown in Table 7A.

Table 7A. VIR5 Nucleotide Sequence (SEQ ID NO:9)

```

ATGGCACTGGCAAAACGCATCATCCCCCTGCCTCGACGTGGACAACGGCCGAGTGGTCAAGGGCGTCA

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AGTTCGAGAACATCCGCGACGCCGGCGACCCGGTTCGAGATCGCTCGCCGCTACGACGAGCAGGGTGC
CGACGAGATCACCTTCCTCGATATCACCGCCAGCGTCGACGGGCGCGACACCACCTGCATACCGTC
GAGCGCATGGCTAGCCAGGTGTTTATTCCGCTGACCGTGGGCGGGCGGTACGCAGCGTGCAGGACA
TCCGCAACCTGTTGAATGCCGGCGCGGACAAGGTCTCGATCAACACCGCCGCGGTGTTCAACCCCGA
GTTTCGTGGGTGAGGCCGCCGACCGCTTCGGCTCGCAGTGCATCGTGGTCCCATCGACGCGAAGAAG
GTTTCCGCCCGGGCGAGGCCGCCGCGCTGGGAAATCTTACCCATGGCGGGCGCAAGCCCACCGGGC
TGGATGCCGTGCTCTGGGCGAAGAAGATGGAAGACTTGGGCGCTGGCGAGATTCTCCTGACCAGCAT
GGACCAGGACGGCGTGAAGAGCGGTTACGACCTGGGCGTGACCCGCGCCATCAGCGAGGCGGTGAAC
GTGCCGGTGATCGCTTCCGGCGGCGTCCGCAACCTGGAGCACCTGGCCGCCGCATCCTCGAGGGCA
AGGCCGACGCGGTGCTCGCGGCGAGCATCTTCCACTTGGCGAGTACACCGTGCCGGAAGCCAAGGC
CTACCTGGCCAGCCGCGGTATCGTGGTGGCTGA

```

The VIR5 protein (SEQ ID NO:10) encoded by SEQ ID NO:9 is presented using the one-letter amino acid code in Table 7B.

Table 7B. Encoded VIR5 protein sequence (SEQ ID NO:10)

```

MALAKRIIPCLDVDNGRVVKGVKFENIRDAGDPVEIARRYDEQGADEITFLDITASVDGRDRTLH
TVERMASQVFIPLTVGGGVRSVQDIRNLLNAGADKVSINTAAVFNPEFVGEAADRFSGQCIIVVAI
DAKKVSAPGEAPRWEIFTHGGRKPTGLDAVLWAKKMEDLGAGEILLTSMQDGVKSGYDLGVTRA
ISEAVNVFVIASGGVGNLEHLAAGILEGKADAVLAASIFHFGEYTVPEAKAYLASRGIVVR

```

MUT6

A *Pseudomonas* bacterial mutant (MUT6) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding N-acetyl-•-glutamyl-phosphate reductase (ArgC; PA0662). This gene encodes the VIR6 nucleic acid (SEQ ID NO:11) shown in Table 8A.

Table 8A. VIR6 Nucleotide Sequence (SEQ ID NO:11)

```

ATGATCAAGGTCGGCATCGTTGGCGGTACGGGTTATACGGGCGTGGAAGTCTGCGCCTGCTGGCGC
AGCATCCGCAGGCCCGGGTGAAGTGATCACTTCGCGTTCGAGGCGGGGGTGAAGGTCGCCGACAT
GTACCCGAACCTGCGAGGTCAATTATGACGACCTGCAGTTCAGCGTGCCGGACGCGCAGCGCCTCGGC
GCCTGCGACGTGGTGTCTTCGCCACGCCGACGGCGTGGCGCACGCGCTGGCTGGCGAAGTCTGG
ACGCCGGGACCCGGGTCATCGATCTGTCCGCTGACTTCCGCCTGGCGGACGCCGAGGAGTGGGCGCG
CTGGTACGGCCAGCCGCATGGCGCTCCGGCGCTGCTCGACGAGGCTGTCTACGGCCTGCCGGAAGTG
AACC GCGAGAAGATCCGCCAGGCCCGCCTGATCGCCGTGCCGGGCTGCTACCCGACCGCAGCCAGC
TGGGCCTGATCCCGCTGCTGGAAGCCGGCCTGGCCGACGCCCTCGCGGCTGATCGCCGATTGCAAGTC
CGGGGTCAGCGGTGCCGGTCCGGGCGCCAAGGTTGGCTCGCTGTCTGCGAGGCGGGCGAAAGCATG
ATGGCCTACGCGGTCAAAGGGCATCGGCATCTCCCGAAATCAGCCAGGGCCTGCGTCCGGGCTCCG
GCGGCGACGTGGGCTGACGTTCTGACCGCACCTGACGCCAATGATCCGCGGTATCCATGCAACCCT
CTATGCCCATGTCGCGGATCGCTCGGTTCGACCTCCAGGCGTTGTTTCGAGAAGCGCTACGCCGAGAA
CCCTTCGTGACGTGATGCCGGCCGGCAGCCATCCGGAGACCCGACGCGTGGTGGCGCCAATGTCT
GCCGAATCGCCGTGATCGCCCCCAGGGCGGGCGACCTGGTGGTGGTGTGTCGGTGATCGACAACCT
GGTCAAGGGCGCCTCGGGTCAGGCGCTCCAGAACATGAACATCTCTGTTGGGCTGGACGAGCGCCTG
GGCCTCTCGCATGCGGCCCTGCTCCCTGA

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The VIR6 protein (SEQ ID NO:12) encoded by SEQ ID NO:11 is presented using the one-letter amino acid code in Table 8B.

Table 8B. Encoded VIR6 protein sequence (SEQ ID NO:12)

MIKVGIVGGTGYTGVELLRLLAQHPQARVEVITSRSEAGVKVADMYPNLRGHYDDLQFSVPDAQRLGACDVVFATPHGVHAHALAGELLDAAGTRVIDLSADFRADAEWARWYQPHGAPALLDEAVYGLPEVNREKIRQARLIAVPGCYPTATQLGLIPLLEAGLADASRLIADCKSGVSGAGRGAKVGSFLCEAGESMMAYAVKGHRHLPETISQGLRRASGGDVGLTFVPHLTPMIRGIHATLYAHVADRSVDLQALFEKRYADEPFVDVMPAGSHPETRSVRGANVCRIAVHRPQGGDLVVVLSVIDNLVKASGQALQNMNILFGLDERLGLSHAALLP

The role of VIR6 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT7

A *Pseudomonas* bacterial mutant (MUT7) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding dihydrolipoamide acetyltransferase (AceF; PA5016). This gene encodes the VIR7 nucleic acid (SEQ ID NO:13) is shown in Table 9A.

Table 9A. VIR7 Nucleotide Sequence (SEQ ID NO:13)

GTGAGCGAACTCATTCGCGTACCCGACATCGGCAACGGTGAGGGTGAAGTCATCGAGCTGCTGGTCAAGCCCGGCGACAAGGTCGAGGCCGATCAGAGCCTGCTGACCCTGGAATCCGACAAGGCCAGCATGGAATCCCCAGTCCCAAGGCCGGGTAGTGAAAAGCATCAAGGCGAAGGTCGGCGACACCTTGAAAGAAAGGTGACGAAATCCTCGAGCTGGAAGTGGAAGGCCGGCGAACAGCCTGCCGAAGCCAAGGCCGAGGCAGCGCCCGCCCAACCGGAAGCGCCGAAAGCCGAAGCGCCTGCTCCCGCCCCGAGCGAGAGCAAGCCGGCCGCCCCCGCGGCCAGCGTCCAGGACATCAAGGTCCCGGACATCGGCTCGGCCGGCAAGGCCAACGTCATCGAAGTGATGGTCAAGGCCGGCGACACGGTCGAGGCCGACCAGTCGCTGATCACCCTGGAATCCGACAAGGCCAGCATGGAGATCCCCCTCGCCGGCCTCCGGGGTGGTGGAAAGCGTCTCGATCAAGGTCGGTGACGAAGTCGGCACCGGCCGACCTGATCCTCAAGCTGAAGGTGGAAGGCCCGCTCCGGCAGCCGAAGAGCAACCGGCAGCCGCTCCGGCCCCAGGCCGCGGCCCGCCGCGGAGCAGAAGCCCGCCGCGCGCGCCCCCTGCGCCAGCCAAGGCCGATACCCCGGCTCCGGTCGGCGCACCCAGCCGCGACGGCGCCAAAGGTCCACGCCGGCCCCGGCGGTGCGCATGCTGGCGCGCAGTTTCGGCGTCGAGCTGAGCGAAGTGAAAGCCAGCGGTCCCAAGGGTCGCATCCTCAAGGAAGACGTCCAGGTCTTCGTCAAGGAGCAACTGCAGCGCGCAAGTCCGGCGGTGCCGGCGCCACCGGCGGAGCCGGCATCCCGCCGATCCCGGAAGTCGACTTCAGCAAGTTCGGCGAAGTGGAAGAAGTGCGCATGACCCGCTGATGCAGGTGCGCGCCGCCAACCTGCATCGCAGCTGGCTGAACGTGCCGCACGTGACCCAGTTTCGACCAAGTCGGACATCACCGACATGGAAGCCTTCCGCGTTGCCCAGAAGGCCGCGCGCGGAGAGGCCGGGTCAAGCTGACCGTACTGCCGATCCTGCTCAAGGCCTGCCGCCACCTGCTCAAGGAAGTGCAGGACTTCAACAGTTTCGCTGGCCCCAGCGGAAGGCGCTGATCCGCAAGAAGTACGTACACATCGGCTTCGCGGTGGACACTCCGGACGGCCTGCTGGTCCCGGTGATCCGCGATGTGACCGGAAGAGCCTCCTGCAACTGGCCGCCGAGGCCGCCGACCTGGCCGACAAGGCCCGCAACAAGAAGCTCTCGGCCGATGCCATGCAGGGCGCCTGCTTACCATCTCCAGTCTCGGCCACATCGGCCGACCGGCTTCACGCCGATCGTCAACGCGCCGGAAGTGCGGATCCTCGGTG

TGTCCAAGGCGACCATGCAGCCGGTATGGGACGGCAAGGCCTTCCAGCCGCGCCTGATGCTGCCGCT
 GTCGCTGTCCTACGACCATCGCGTGATCAACGGTGCCGCCGCGCGCTTCACCAAGCGCCTGGGC
 GAGCTGCTGGCGGACATCCGCACCCTGCTCCTGTAA

The VIR7 protein (SEQ ID NO:14) encoded by SEQ ID NO:13 is presented using the one-letter amino acid code in Table 9B.

Table 9B. Encoded VIR7 protein sequence (SEQ ID NO:14)

MSELIRVPDIGNGEGEVIELLVKPGDKVEADQSLLTLESDKASMEIPSPKAGVVKSIKAKVGD
 KEGDEILELEVEGGEQPAEAKAEAAPAQPEAPKAEAPAPAPSESKPAAPAAASVQDIKVPDIGSA
 GKANVIEVMVKAGDTVEADQSLITLESDKASMEIPSPASGVVESVSIKVGDEVGTGDLILKLKVE
 GAAPAAEEQPAAPAAQAAAPAAEQKPAAPAPAKADTPAPVGAPSRDGAKVHAGPAVRMLAREF
 GVELSEVKASGPKGRILKEDVQVFVKEQLQRAKSGGAGATGGAGIPPIPEVDFSKFGEVEEVAMT
 RLMQVGAANLHRSWLNVPVHTQFDQSDITDMEAFRVAQKAAAEKAGVKLTVLPILLKACAHLLKE
 LPDFNSSSLAPSGKALIRKKYVHIGFAVDTPDGLLVPIRDVDRKSLQLAAEAADLADKARNKKL
 SADAMQGACFTISSLGHIGGTGFTPIVNAPEVAILGVSKATMQPVWDGKAFQPRMLPLSLSYDH
 RVINGAAAARFTKRLGELLADIRTL

MUT8

A *Pseudomonas* bacterial mutant (MUT8) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding NADH dehydrogenase I chain H (nuoH; PA2643). This gene encodes the VIR8 nucleic acid (SEQ ID NO:15) shown in Table 10A.

Table 10A. VIR8 Nucleotide Sequence (SEQ ID NO:15)

ATGAGTTGGCTGACTCCCGCTCTGGTCACCATCATCCTCACCCTGGTCAAGGCCATCGTGGTGCTGC
 TCGCCGTGGTCATCTGCGGCGCCCTGCTAAGCTGGGTCGAGCGCCGCTGCTCGGCCCTCTGGCAGGA
 CCGCTACGGCCCCAACCGGGTCGGTCCGTTCCGTTGCGTTCAGCTCGGCGCGGACATGGTCAAGATG
 TTCTTCAAGGAGGACTGGACCCCGCCGTTCCGCCGACAAGATGATCTTCACCTTGCCCCCGGTAATCG
 CGATGGGCGCCCTGCTCGTCGCCCTCGCCATCGTGGCGATCAGCCACCTGGGGCGTGGCGGACCT
 GAACATCGGCATCCTGTTCTTCTTCGCCATGGCCGGCCTGACGGTGTACGCCGTGCTGTTCGCCGGC
 TGGTCGAGCAACAACAAGTTCCGCCCTGCTCGGCAGCCTGCGCGCCTCGGCCAGACCATCTCCTACG
 AGGTGTTCTGGCCCTGTCGCTGATGGGCATCGTCCGCCAGGTGCGCTCGTTCAACATGCGCGACAT
 CGTCCAGTACCAGATCGACAACGTCTGGTTCATCATTCGCGAGTTCTTCGGCTTCTGCACCTTCATC
 ATCGCCGGCGTCGCCGTGACCCACCGTCACCCGTTGCGACAGCCGGAAGCGGAGCAGGAAGTGGCGG
 ACGGCTACCACATCGAGTACGCCGGGATGAAATGGGGCATGTTCTTCGTCGGCGAGTACATCGGCAT
 CGTACTGGTCTCGGCGCTGCTGGCGACCTGTTCTTCGGCGGCTGGCACGGTCCGTTCTGGACACC
 CTGCCCTGGCTGTCGTTCTTCTACTTCGCCGCCAAGACCGGCTTCTTCATCATGCTCTTCATCCTGA
 TCCGCGCCTCGCTGCCGCGTCCGCGCTATGACCAGGTGATGGCGTTTACGCTGGAAGGTGTGCGCTGCC
 GCTGACCCTGATCAACCTGCTGGTGACCGCGCGCTCGTGTGGCCGCGGCCAGTAA

The VIR8 protein (SEQ ID NO:16) encoded by SEQ ID NO:15 is presented using the one-letter amino acid code in Table 10B.

Table 10B. Encoded VIR8 protein sequence (SEQ ID NO:16)

MSWLT PALVTI ILTVVKAIVVLLAVVICGALLSWVERRLGLWQDRYGPNRVGPFGAFQLGADMV
 KMFFKEDWTPPFADKMI FT LAPVIAMGALLVAF AIVPITPTWGVADLNIGILFFFAMAGLTVYAV
 LFAGWSSNNKFALLGSLRASAQTISYEVFLALSLMGIVAQVGSFNM RDIVQYQIDNVWF IIPQFF
 GFCTFTIAGVAVTHRHPFDQPEAEQELADGYHIEYAGMKWGMFFVGEYIGIVLVSALLATLFFGG
 WHGPF LDTLPWLSFFYFAAKTGFFIMLFILIRASLPRPRYDQVMAFSWKVCLPLTLINLLVTGAL
 VLAAAQ

5 MUT9

A *Pseudomonas* bacterial mutant (MUT9) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding pyoverdine synthase D (PvdD; PA2399). This gene encodes the VIR9 nucleic acid (SEQ ID NO:17) shown in Table 11A.

Table 11A. VIR9 Nucleotide Sequence (SEQ ID NO:17)

GTGCAAGCACTCATAGAGAAGGTGGGCTCCCTTTCCCCCAGGAAAGGAAGGCATTGGCTGTCTCTGC
 TCAAGCAGCAAGGTGTCAATCTCTTCGAGATCGCGCCAGTGTTCAAGCGCCAGGACGGCGAGCCCCCT
 GCGGCTCTCCTATGCCCCAGGAGCGACAGTGGTTCTCTGGCAACTGGAGCCGGAACGCGGGCTTAC
 CATATCCCCGAGTGCTCTGCGCTCTACGTGGGCGGGCTGGACCTGGATGCCCTGCAACGCAGCTTCGACA
 GCCTGGTTGCGCGGCACGAGACCCCTACGCACCCGTTTTCGCCCTCGACGGCGACGAGGCGCGCCAGGA
 GATCGCCGCATCCATGGCATTGCCGTTGGATATCGTCGCGTTGGGGCCGCTCGAGGAGGGCGCCCTC
 GCTCGGCAGGTCGAGACGACGATCGCGCGGGCGGTTTCGACCTGGAGCGTGGGCGGCTGCTGCGGGTGA
 GCCTGTTGCGGCTGGCCGAGGACGACCATGTGCTGGTGTGGTCCAGCATCACATCGTGTCCGACGG
 TTGGTTCGATGCAGGTGATGGTTCGAGGAACGTGGTCCAGCTCTATGCCGCCATAGTCGAGGGCTCGAG
 GTAGCGTGTCCGCTTTGCCGATCCAGTACGCGGACTACGCCCTGTGGCAGCGCAGCTGGATGGAGG
 CCGGGGAAAAGGAGCGCCAGTTGGCGTACTGGACCGGCTGCTGGGCGGCGAGCAGCCGGTGCTGGA
 GTTGCCGTTTCGACCGGCCGCGCCCGGTTTCGGCAAAGCCATCGTGGTGCCAGTTTCATCCTGGAACGTG
 GATATTGATCTGTCCCAGGCGCTCAGGCGCGTGGGCCAGCAGGAGGGGGCTACTGCCTTCGCCCTGT
 TGCTGGCTTCGTTCCAGGCGCTGCTGTATCGCTACAGCGGGCAGGCGGATATCCGTGTGCGCGTGCC
 GATCGCCAATCGCAACCGCGTGGAGACCGAGCGGCTGATCGGCTTCTTCGTCAACACCCAGGTGCTC
 AAGGCCGACCTGGACGGTCCGATGGGCTTCGACGAGCTGCTGGGCCAGGCCCGCCAACGCGCGCTGG
 AGGCCAGGCGCACAGGACCTGCCGTTTCGAGCAACTGGTGGAGGCCCTTCAGCCGGAGCGCAGTCT
 TAGCCACAACCCGCTGTTCAGGTGCTGTTCAACTACCAGAGCGAAGCCCGTGGCAACGGCCAGGCA
 TTCCGCTTCGACGAGTTACAGATGGAAGCGTGCAAGTTTCGACAGCCGGACGGCGCAGTTTCGACTTGA
 CGTTGGACCTGACGGACGAAGAGCAGCGTTTTTTCGCGCGTTTTCGACTACGCCACCGACCTGTTTGA
 CGCCTCCACCGTGGAAACGCTTGGCCGGCCATTGGCGCAACCTGTTGCGCGGCATCGTCGCCAACC
 CGACAGCGGCTCGGCGAGTTGCCGCTGCTGGATGCGCGGAGCGCCGGCAGACCCCTCTCCGAATGGA
 ACCCGGCCAGCGGAGTGCGCGGTGACGGCACCTTCGAGCAGCGTTTCGAGGAACAGGCGCGGCA
 ACGGCCACAGGCGGTTGCGCTGATCCTCGACGAACAACGGTTGAGCTACGGCGAAGTGAATGCGCGG
 GCCAATCGCCTGGCGCACTGCCTGATCGCCCGTGGCGTTGGCGCGGACGTGCCGGTTCGGGCTGGCGC
 TGGAGCGTTTCGCTGGACATGCTGGTGGCTTGTGGCGATCCTCAAGGCCGGCGGCGCCTACCTGCC
 GTTGGACCCGGCGGCGCCAGAGGAGCGCTGGCGCATATCCTCGACGACAGTGGGGTACGGCTGCTG
 CTGACCCAGGGGCGATGCTCGAGCGCTGCCACGGCAGGCGGGGTTGGAGGTGCTGGCCATCGACG
 GACTGGTGTGTCGACGGCTACGCCGAGAGCGATCCGCTCCCGACGCTATCGGCGGACAACCTGGCCTA

CGTGATCTATACCTCGGGCTCGACCGGCAAGCCCAAGGGCACATTGCTCACCACCGCAACGCGCTG
 CGCCTGTTTCAGCGCCACCGAGGCTGGTTTCGGCTTCGACGAGCGGGACGTGTGGACATTGTTCCATT
 CCTACGCCCTTCGATTTCCTCGGTCTGGGAAATCTTCGGCGCGCTGCTCTATGGCGGGTGCCTGGTGAT
 TGTGCCGCAATGGGTGAGCCGTTCGCCGGAAGACTTCTACCGTCTGCTGTGCCGGAAGGCGTGACG
 GTGCTCAACCAGACGCCGTGCGCTACGTGATCTTCGGTGGCGAGGCGCTGGATCTGCAGAGCCTGCGGCC
 CGCAGCAGCGCGGCTGCGCTACGTGATCTTCGGTGGCGAGGCGCTGGATCTGCAGAGCCTGCGGCC
 GTGGTTCCAGCGCTTCGGCGATCGCCAGCCGCAACTGGTGAACATGTACGGCATEACCGAGACCAGG
 GTGCACGTAACCTACCGTCCGGTGAGCGAGGCGACCTGGAAGGTGGCCTGGTCACTCCGATTGGCG
 GGACCATCCCGGACCTGTCTGGTACATCTCGACCGTGACCTGAACCCGCTGCCGCGCGCGCGGT
 GGGCGAGCTGTACATCGGTGCGCGCGGGCTGGCGCGCGGTACCTGAGGCGGCCCGGGTTGAGTGCC
 ACCCGCTTCGTGCCGAACCCGTTCGCCGCGCGCGCGCGGCGAGCGGCTGTACCGTACCGGCGACCTGG
 CACGGTTCCAGGCGGATGGCAATATCGAGTACATCGGGCGTATCGACCACAGGTGAAGGTTTCGCGG
 CTTCCGTATCGAACTGGGCGAGATCGAAGCGGCGCTCGCCGGTCTGGCCGGGGTACGCGATGCCGTG
 GTGCTGGCCCCATGACGGAGTTCGGCGGCACGCAACTGGTGGGATACGTGGTGGCGGACTCGGCGGAGG
 ATGCCGAGCGTCTGCGGGAGTTCGTGCGGGAGTTCGTGAAGCGGCACCTGCCGGAACATCGGTGGC
 GGGCGACCTGATGCTGCTGGAGCGGATGCCGCTGACGGTCAATGGCAAGCTCGACCGGCGAGGCGTTG
 CCGCAACCGGATGCGAGCCTGTGCAACAGGCGTATCGAGCGCGCGGTAGCGAGCTGGAGCAGCGCA
 TCGCAGCGATCTGGTTCGGAGATCCTGGGAGTGGAAACGGGTTCGGCTGGACGACAACCTTCTTCAACT
 GGGCGGTTCATTCTGCTGGCTACCCGGGTGATTTCTCGGGTTCGCCAGGAGCAGCTGGAGCGCA
 AGCCTGAAGGCGTTGTTTCGAGCGGCGGTTCTGGAAGCGTTTCGCCAGGGATTGGAACGCACGACGG
 ATGCGGTCTCGACGATACCGCTTGGCGATCGGCAGCAACCGTTGGCACTGTCTTTCGCTCAGGAGCG
 TCAGTGGTTCTCTGGAACCTGGAGCCGGAAGCGCGGCTTACCATATTCGAGTGCCTTTCGCGCTA
 CGCGGCGGCTGGACGTGGATGCCCTGCAACGCAGCTTCGACAGCCTGGTTCGCGCGGCGATGAAACCT
 TGGCTACCCGCTTCGGCTGGAGGGAGGGCGTTCGTACAGCAGGTACAACCTGCGGTTAGCGTTTC
 CATCGAGCGGGAACAGTTTCGGTGAAGAAGGCGTTCGCAACGGATACAGGCCATCGTTGTGACGCCA
 TTCGACCTGGAACGGGGCGGCTGCTGCGGGTGAACCTGTTGCAACTGGCCGAGGACGACCATGTAC
 TGGTCTGTTCCAGCACCATCTGTTCCGATGGTTGGTTCGATGCAGGTGATGGTTCGAGGAACCTGGT
 CCAGCTCTATGCCGCTATAGCCAAGGGCTCGACGTGGTGTGTCAGCCCTGCCGATCCAGTACGCG
 GACTACGCCCTGTGGCAGCGCAGCTGGATGGAGCGGGGAAAAGGAGCGCCAGTTGGCGCTACTGGA
 CCGGCTGCTGGGCGGCGAGCAGCCGGTGTGCTGGAGTTGCCGTTTCGATCGGCGCGCTCGGCGCGGCA
 GAGCATCGTGGCGCGCAGTTGGGTTTCGAGCTATCGCGGGAACCTGGTTCGAGGCGGTGAGAGCCTTG
 GCCAGCGTGAAGGCGCCAGTAGTTTCATGCTGTTGCTGGCCTCGTTCCAGGCGCTGTTGTATCGCT
 ACAGCGGCGAGGCGGATATCCGTGTCGGTGTGCGGATCGCCAATCGCAACCGCGTGGAGACCGAGCG
 GCTGATCGGCTTCTTCGTCAACACCCAGGTGCTCAAGGCGACCTGGACGGTCCGATGGCGTTTCGAC
 GAGCTGCTGGCCAGGCGCGCAACCGCGCGCTGGAGCGCCAGGCGCACAGGACCTGGCGTTTCGAGC
 AACTGGTGAAGCCTTCGAGCCGGAGCGCAATGCCAGCCACAACCCACTGTTCCAGGTGCTGTTCAA
 CCATCAGAGCGAGATACGCTCGGTGACGCGCCGAGGTTTCAGTTGGAGGACCTGCGCTTCGGAAGGCGTG
 GGCTGGGACGGCCAGACTGCGCAGTTTCGACCTGACGCTGGATATTCAGGAAGACGAAAACGGCATCT
 GGGCTTCCTTCGACTATGCCACCGATCTGTTGACGCTCCACCGTGGAAACGCTGGCGCGGCAATTG
 GCGCAACCTGTTGCGCGCATCGTCGCCAACCCAGCAGCGGCTCGGCGAGTTGCGGCTGCTGGAT
 GCGCGGAGCGCGGCGAGACCTCTCCGAATGGAACCCGCGCCAGCGGAGTGGCGGTTGCGGCGGCA
 CCTTGCAGCAGCGTTTCGAGGAGCAGGCGCGCAACCGCCACAGGCGGTTGCGCTGATCTTCGACGA
 ACAACGGTTGAGCTACGGCGAACTGAATGCGCGGGCAATCGCCTGGCGCACTGCGCTGATCGCTCGC
 GCGCTTGGCGCGGAGTGGCGGCTGGCGGCTGGAGCGTTTCGCTGGACATGCTGGTGGCTGGCTGGC
 TGGCGATCTCAAGGCGGCGGCGCTTACCTGCCGTTGGACCGCGCGCGGAGAGCGGCTGGC
 GCATATCTTCGACGACAGTGGGTACGGCTGCTGCTGACCCAGGGGCGATCTGCTCGAGCGCTGCCG
 CGGCGAGGCGGGGTGGAGGTGCTGGCCATCGACGGACTGGTGTGACGGCTACGCCGAGAGCGATC
 CGCTCCCGACGCTATCGGCGGACAACCTGGCCTAGCGTGATCTATACCTCGGGCTCGACCGGCAAGCC
 CAAGGCGACGTTGCTACCCACCGCAACGCGCTGCGCTGTTTCAGCGCCACCGAGGCGTGGTTCCGGC
 TTCGACGAGCGGGACGTGTGGACGTTGTTCCATTCTTACGCTTCGATTTCTCGGTCTGGGAAATCT
 TCGGCGCGCTGCTCTATGGCGGGCGCTGGTGTGCTGCGCAATGGGTGAGCCGTTTCGCCGGAAGA
 CTTCTACCGTCTGCTGTGCCGCGAAGGCGTGACGGTGTCAACAGACGCCGTCGGCGTTCAAGCAA
 CTGATGGCGGCTGGCTGTTCCGCCGACATGGCGAGCAGCAGCGGCGCTGCGCTACGTGATCTTCG
 GTGGCGAGGCGCTGGATCTGCAGAGCCTGCGGCGGTGGTTCCAGCGCTTTGGCGATCGCCAGCCGCA
 ACTGGTGAACATGTACGGCATCACCGAGACCAGGTACACGTAACCTACCGTCCGGTGAGCGAAGCC
 GACCTGAAGGTTGGCTGGTCACTCCGATCGGCGGGACCATCCCGGACCTGTCTGGTACATCTTCG
 ACCGTGACCTGAACCCGGTGGCGCGCGGCGCGGTGGGCGAGCTGTACATCGGTGCGCGCGGTCTGGC
 GCGGCGCTACCTGAGGCGGCGCGGCTTGGTGGCACCCGCTTCGTGCGGAACCCGTTCCCGCGCGGT
 GCGGCGAGCGGCTGTACCGTACCGGCGACCTGGCACGCTTCAGGCGGATGGCAATATCGAGTACA
 TCGGGCGTATCGACACCAGGTGAAGGTTTCGCGGCTTCGCTATCGAACTGGGTGAGATCGAAGCGG
 GCTCGCGGCTGCGCGGGGTACGCGATGCCGTTGGTGTGGCCATGACGGGGTGGCGGCGACGCAA
 CTGGTGGGATACGTGGTGGCGGACTCGGCGGAGGATGCCGAGCGTCTGCGGGAGTCTGCGGGAGT
 CGCTGAAGCGGACCTGCCGACTACATGGTGGCGGCGACCTGATGCTGCTGGAGCGGATGCCGCT
 GACGGTCAATGGCAAGCTCGACCGGCGAGGCTGGCGCAACCGGATGCGAGCTTGTGCGAGCAGGCC
 TATCGAGCGCCGGTAGCGAGCTGGAGCAGCGCATCGCAGCGATCTGGGCGGAGATCCTGGGAGTGG

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AACGGGTCGGCCTGGACGACAACCTTCTTCGAACTGGGCGGTCACTCATTGTTGCTGCTGATGCTCAA
GGAGCGGATCGGCGATACCTGCCAGGCTACGCTGAGCATCAGCCAACCTGATGACCCATGCCAGCGTC
GCGGAACAGGCGGCATGCATCGAGGGGAGGCGCGTGAGTCGTTGCTGGTGCCGCTCAACGGCAGGC
GCGAAGGTTCCGCGCTGTTTCATGTTCCATCCGAGTTTCGGCTCTGTGCACTGTTACAAGACCCCTCGC
CATGGCGCTGCGGGATCGTCATCCGGTCAAGGGTGTTGTCTGCCGTGCCCTGCTGGGCGCTGGTTCGC
GAGGTGCCGGAGTGGGACGATATGGTTGCGGAATACGCCGAGCAATTGCTGCAGGAGCACCCTGAAG
GGGTTTTCAACCTGGCGGGATGGTCGCTCGGCGGCAACCTGGCGATGGATGTCGCGGCCCCGGCTGGA
GCAGCGTGGGCGGAGGTGGCTTTTCGTCGGCTGGATCGATGCACCGGCACCGGTCAGGGTCGAAGCG
TTCTGGAACGAGATCGGGCCGACGCCGGAGGCAGTCCCGAACCTATCCGTGGGCGAGATGCGGGTGG
AATGCTCGGTGTCATGTTTCCGGAGCGGGCCGAGCATATCGAACGGGCTGGTCATCGATCTGCTC
CGCCACGACGGACGATGAGCAGCGCTGGACGAGGATGAGCGACTGGGCGGAAGCGGAGATCGGGCGCC
GAGTTCGCGACACTGCGCAGCGAAATCGCACAGAGCAACGAACGGAAGTGCTCTGGGAGTTGAAAC
AGATCCTCGACGAGCGCCTGAAAGCGATGGATTACCGCGCTCTGACGGCGAAGGTGAGCCTCTGGTG
GGCCGCGCGCAGCACCATGCCATCCAGCGGAGCGCGGTGGAGCGCTCGATGGCCGAGGCGATCGGG
GCTGAGCGTGTGCAACCGGTGCGGGTGCTGGATACCGGCACGACAAGATCATCGACCACCTGAGT
TTGTGCAGAGCTTCCGGGCGGCCCTGGAGCGTGCCGGGCGCTGA

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The VIR9 protein (SEQ ID NO:18) encoded by SEQ ID NO:17 is presented using the one-letter amino acid code in Table 11B.

Table 11B. Encoded VIR9 protein sequence (SEQ ID NO:18)

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MQALIEKVGSLSPQERKALAVLLKQQGVNLFETAPVFKRQDGEPLRLSYAQERQWFLWQLEPESA
AYHIPSVLRLRGRDLDALQRSFDSLVARHETLRTFRRLDGEARQETAAASMLPLDIVALGPLE
EGALARQVETTIARPFDLERGPLLRLVSLRLAEDDHVLLVQHHIVSDGWSMQVMVEELVQLYAA
YSRGLEVALPALPIQYADYALWQRSWMEAGEKERQLAYWTGLLGGEQPVLELPFDRPRPVQRSHR
GAQFLELDIDLSQLRRVAQEGATAFALLASFAQALLYRYSQADIRVGVPIANRNRVETERL
IGFFVNTQVLKADLDGRMGFDELLAQARQRALEAQAHQDLPEQLVEALQPERSLSHNPLFQVLF
NYQSEARGNGQAFRDELQMESVQFDSRTAQFDLTLDLTDDEQRFCVFDYATDLFDASTVERLA
GHWNRNLLRGIVANPRQRLGELPLLDAPERRQTLSEWNPAQRECAVQGTQQRFEEQARQRPQAVA
LILDEQRLSYGELNARANRLAHCLIAARGVGADVPVGLALERSLDMVLVGLLAILKAGGAYLPLDPA
APEERLAHILDDSGVRLLLTQGHLLERLPRQAGVEVLAIDGLVLDGYAESDPLPTLSADNLAYVI
YTSGSTGKPKGTLLTHRNALRLFSATEAWFGFDERDVTWTLFHSYAFDFSVWEIFGALLYGGCLVI
VPQWVSRSPEDFYRLLCREGVTVLNQTPSAFKQLMAVACSADMATQQPALRYVIFGGEALDLQSL
RPWFQRFQDRQPOLVNMYGITETTTHVHTYRVPVSEADLEGGLVSPIGGTIPDLWSYILDRDLNPV
RGAVGELYIGRAGLARGYLRRPGLSATRFVNPFPFGGAGERLYRTGDLARFQADGNIEYIGRIDH
QVKVRGFRIELGEIEAALAGLAGVRDAVVLAHGCVGGTQLVGVVADSADAEERLRESLRESLKR
HLPDYMVPAHMLLERMPLTVNGKLDLQALPQPDASLSQAYRAPGSELEQRIAAIWSEILGVER
VGLDDNFFELGGHSLLATRVISRVRQEQQLDASLKALFERPVLFAAQGLERTTDAVSTIPLADR
QQPLALSFAQERQWFLWQLEPESAAHYIPSAALRLRGRDLVDALQRSFDSLVARHETLRTFRLEG
GRSYQQVQPAVSUSIEREQFGEEGLIERIQAIVVQPFDLERGPLLRLVNLQLAEDDHVLLVQHH
IVSDGWSMQVMVEELVQLYAAYSQGLDVLPALPIQYADYALWQRSWMEAGEKERQLAYWTGLLG
GEQPVLELPFDRPRPARQSHRGAQLGFELSRELVEAVRALAQREGASSFMLLLASFAQALLYRYSQ
QADIRVGVPIANRNRVETERLIGFFVNTQVLKADLDGRMGFDELLAQARQRALEAQAHQDLPEEQ
LVEALQPERNASHNPLFQVLFNHQSEIRSVTPEVQLEDLRLLEGLAWDGQTAQFDLTLDIQEDENG
IWASFDYATDLFDASTVERLAGHWNRNLLRGIVANPRQRLGELPLLDAPERRQTLSEWNPAQRECA
VQGTQQRFEEQARQRPQAVAILDEQRLSYGELNARANRLAHCLIAARGVGADVPVGLALERSLD
MLVGLLAILKAGGAYLPLDPAPEERLAHILDDSGVRLLLTQGHLLERLPRQAGVEVLAIDGLVL
DGYAESDPLPTLSADNLAYVIYTSGSTGKPKGTLLTHRNALRLFSATEAWFGFDERDVTWTLFHSY
AFDFSVWEIFGALLYGGRLVIVPQWVSRSPEDFYRLLCREGVTVLNQTPSAFKQLMAVACSADMA
TQQPALRYVIFGGEALDLQSLRPWFQRFQDRQPOLVNMYGITETTTHVHTYRVPVSEADLKGGLVSP
IGGTIPDLWSYILDRDLNPVPRGAVGELYIGRAGLARGYLRRPGLSATRFVNPFPFGGAGERLYR
TGDLARFQADGNIEYIGRIDHQVKVRGFRIELGEIEAALAGLAGVRDAVVLAHGCVGGTQLVGVY
VADSADAEERLRESLRESLKRHLPDYMVPAHMLLERMPLTVNGKLDLQALPQPDASLSQAYRAP
PGSELEQRIAAIWAEILGVERVGLDDNFFELGGHSLLLMLKERIGDTCQATLSISQLMTHASVA
EQAACIEGQARESLLVPLNGRREGSPFLFMFHPFSGSVHCHYKTLAMALRDRHPVKGVVCRALLGAG
REVPEWDDMVAEYAEQLLQEHPEGVFNLAGWSLGGNLMAMDVAARLEQRGRQVAFVGVWIDAPAPVR
VEAFWNEIGPTPEAVPNLSVGEVRVLLGVMPFERAEHIERAWSSICSATTDDEQRWTRMSDWAEE
AEIGAFAFATLRSEIAQSNELEVSWELKQILDERLKAMDYPRLTAKVSLWWAARSTNAIQRSAVER
SMAEIGAERVEPVRVLDTRHDKIIDHPEFVQSFRAALERAGR

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MUT10

A *Pseudomonas* bacterial mutant (MUT10) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding the RND multidrug efflux transporter MexD (mexD; PA4598). This gene encodes the VIR10 nucleic acid (SEQ ID NO:19) shown in Table 12A.

Table 12A. VIR10 Nucleotide Sequence (SEQ ID NO:19)

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ATGTCCGAATTCTTCATCAAGCGGCCGAACCTTCGCCTGGGTGGTGGCCCTGTTTCATCTCCCTGGCCG
GCCTGCTGGTCATTTCCAAATTGCCGGTAGCGCAGTACCCCAATGTCGCGCCGCCACAGATCACCAT
CACCGCCACCTATCCCGGCGCCTCGGCGAAGGTGCTGGTGGACTCCGTCACCAGTGTGCTCGAGGAG
TCGCTGAACGGCGCCAAGGGCCTGCTCTACTTCGAGTCGACCAACAACCTCCAACGGCACCGCCGAGA
TCGTCGTCACCTTCGAGCCGGGCACCGATCCGGACCTGGGCCAGGTGGACGTGCAGAACCGCCCTGAA
GAAAGCCGAGGCGCGCATGCCGAGGCGGTGCTGACCCAGGGCCTGCAGGTGAGCAGACCAGCGCC
GGTTTCCTGCTGATCTATGCGCTCAGCTACAAGGAAGGCGCTCAGCGCAGCGACACCACCGCCCTCG
GCGACTACGCCGCGCGCAATATCAACAACGAGCTGCGGCGCCTGCCGGGCGTCCGCAAGCTGCAATT
CTTCTCTTCCGAGGCGGCCATGCGGGTCTGGATCGATCCGAGAAAGCTGGTGGGCTTCGGCCTCTCC
ATCGACGACGTGAGCAATGCCATCCGCGGGCAGAACGTGCAGGTGCCGGCCGGCGCCTTCGGCAGCG
CACCGGGCAGTTCCGCGCAGGAGCTGACGGCGACCTTGGCGGTGAAGGGCACCTGGACGATCCGCA
GGAGTTCGGCCAGGTAGTGCTGCGCGCCAACGAGGACGGCTCGCTGGTCCGGCTCGCCGATGTCCGC
CGCTTGAACTCGGCAAGGAGAGCTACAACATTTCTTCGCGACTGAACGGCACGCCACCGTGGGCG
GGGCTATCCAGCTGTGCGCCGGGGCCAACCGGATCCAGACCGCTACCTTGGTGAACAGCGTCTCGC
CGAAGTGTGCGCGTTCTTCCCGGAGGACATGCAGTACAGCGTGCCCTACGACACCTCGCGCTTCGTC
GACGTGGCCATCGAGAAGGTGATCCACACCTGATCGAAGCGATGGTCCCTGGTGTTCCTGGTGTATGT
TCCTGTTCCTGCGAAGCTCCGCTACACCTGATCCCGTCCATCGTGGTGGCGGTGTGCTGCTGGG
TACGCTGATGGTGTATGATCTGCTGGGGTTCTCGGTGAACATGATGACCATGTTTCGGCATGGTCTG
GCGATCGGCATCTTGGTGGACGACGCCATCGTGGTGGTGGAGAACGTCGAGCGGATCATGGCGGAGG
AGGGGATTTCCCCGGCCGAGGCCACGGTCAAGGCGATGAAGCAGGTATCCGGCGCCATCGTCGGCAT
CACCTGGTGTCTTCGGCGGTGTTCTTCCGCTGGCTTTTCATGGCCGGTTCGGTGGGGGTGATCTAC
CAGCAGTTCTCGGTGCTGCGCCACGCTGCTCAAGCCCATTTCCGAAGGGCACACGAGAAGCGCGCTTCTTCGG
CGCTTCAACCGTGGCTTCGCGCGCTCACCGAGCGCTATTTCGCTGCTCAACTCGAAGCTGGTGGCG
CGCGCCGGACGCTTCATGCTGGTGTACGCCGGCTTGGTGGCCATGCTCGGCTACTTCTACCTGCGCC
TGCCGGAAGCCTTTCGTGCGCGGGAAGACCTCGGCTACATGGTGGTTCGACGTGCAACTGCCGCCCTGG
CGCTTCGCGCGTTCGCGACCGATGCCACCGCGAGGAGCTCGAGCGCTTCTCAAGTCCCGCGAGGCG
GTGGCTTCGGTGTTCCTGATCTCGGGCTTCAGCTTCTCCGCGCAGGGCGACAATGCCGCGCTGGCCT
TCCCAACCTTCAAGGACTGGTCCGAGCGAGGCGCCGAGCAGTCGGCCGCCGCCGAGATCGCCGCGCT
GAACGAGCATTTTCGCGCTGCCCGACGATGGCACGGTCAATGGCCGTGTGCGCGCCACCGATCAACGGT
CTGGGTAACCTCCGGCGGCTTCGCAATGCGCCTGATGGACCGTAGCGGGGTTCGGCCGCGAAGCGCTGC
TGCAGGCTCGCGATACTCTTCTTGGCGAGATCCAGACCAACCCGAAATTCCTTTACGCGATGATGGA
AGGACTGGCCGAAGCGCCGCAACTGCGCCTGTTGATCGACCGGGAGAAGGCCCTGCCCTGGGGGTG
AGCTTCGAGACCATCAGCGGCACGCTGTCCGCTGCCTTCGGCTCGGAGGTGATCAACGACTTCACCA
ATGCGGGGCGCCAACAGCGGGTGGTGTATCCAGGCCGAACAGGGCAACCGGATGACCCCGAAAGCGT
GCTCGAGCTATACGTGCCTAACGCTGCTGGCAACCTGGTACCGCTCAGCGCCTTCGTCAGCGTGAAA
TGGGAAGAGGGACCGGTGCAATTGGTGCCTATAACCGCTACCCGTCGATCCGATCGTCGGTGACG
CCGCGCCCGGCTTCAGTACCGGCGAAGCCATGGCGGAAATGGAGCGCCTGGCCTCGCAGCTGCCGGC
CGGCATCGGCTACGAGTGGACCGGCTGTCTATCAGGAGAAGGTCTCCGCGGGGAGGCCACAGC
CTGTTCCGCCCTCGCCATCTGGTGGTGTCTTCTGTTGCTGGTGGCGCTCTACGAGAGCTGGTCGATCC
CGTGTGCGGTGATGCTGATCGTGCCGATCGGCGCCATCGGCGCGGTGCTCGCGGTGATGGTCAGCGG
TATGTCCAACGACGTGTATTTCAAGGTGCGCTATACCGCTACCATCATCGGTCTTCGGCGAAGAACGCG
ATCCTCATCGTCGAGTTCGCCAAGGAACCTCTGGGAGCAGGGGCATAGCCTGCGCGACGCCGATCG
AGGCCGCGCGCCTGCGCTTCCGGCCGATCATCATGACTTCCATGGCGTTTCATCCTCGGCGTGATACC
CCTGGCCCTGGCCAGCGGTGCCGGCGCGGCGAGCCAGCGTGCCATCGGCACCGGAGTGATCGGCGGG
ATGCTCAGCGCCACCTTCTTCGGCGTGCTGTTTCGTACCTATCTGTTTCGTCTGGCTGCTGTGCTGCTGC

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TGCGCAGCAAGCCGGCACCCATCGAACAGGCCGCTTCGGCCGGGGAGTGA

The VIR10 protein (SEQ ID NO:20) encoded by SEQ ID NO:19 is presented using the one-letter amino acid code in Table 12B.

Table 12B. Encoded VIR10 protein sequence (SEQ ID NO:20)

MSEFFIKRPNFAWVVALFISLAGLLVISKLPAQYPNVAPPQITITATYPGASAKVLVDSVTSVL
EESLNGAKGLLYFESTNNSNGTAEIVVTFEPGTDPLAQVDVQNRLLKAEARMPQAVLTQGLQVE
QTSAGFLLIYALSYKEGAQRSDTTALGDYAARNINNELRRLPGVGKLQFFSSEAMRVWIDPQKL
VGFGLSIDDVSNARQNVQVPAGAFGSAPGSSAQELTATLAVKGTLDDEPQVFGQVVLNANEDGS
LVRLADVARLELGKESYNISSRLNGTPTVGGAIQLSPGANAIQTATLVKQRLAELSAFFPEDMQY
SVPYDTSRFVDVAIEKVIHTLIEAMVLVFLVMFLFLQNVRYTLIPSIIVVPVCLLGTLMVMYLLGF
SVNMTMFGMVLAIIGILVDDAIVVVENVERIMAEEGISPAEATVKAMQVSGAIVGITLVLSAVF
LPLAFMAGSVGVYQQFSVSLAVSILFSGFLALTFPALCATLLKPIPEGHHEKRGFFGAFNRGF
ARVTERYSLLNSKLVARAGRFLMVLVYAGLVAMLGIFYLRLPEAFVPAEDLGYMVDVQLPPGASRV
RTDATGEELERFLKSREAVASVFLISGFSFSGQGDNAALAFPTFKDWSERGAEQSAAAEIAALNE
HFALEDDGTVMASVPPPINGLNSGGFALRLMDRSGVGREALLQARDTLLGEIQTNPKFLYAMME
GLAEAPQLRLLIDREKARALGVSFETISGTLAAFGSEVINDFTNAGRQQRVVIQAEQGNRMTP
SVLELYVPNAAGNLVPLSAFVSVKWEEGPVQLVRYNGYPSIRIVGDAAPGFSTGEAMAEMERLAS
QLPAGIGYEWGTGLSYQEKVSAGQATSLFALAILVVFLLLVALYESWSIPLSVMLIVPIGAIGAVL
AVMVSGMSNDVYFKVGLITIIIGLSAKNAILIVEFAKELWEQGHSLRDAIEAARLFRPIIMTSM
AFILGVIPLALASGAGAASQRAIGTGVIIGMLSATFLGVLFVPICFVWLLSLLRSKPAPIEQAAS
AGE

MUT11

A *Pseudomonas* bacterial mutant (MUT11) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding PA3721. This gene encodes the VIR11 nucleic acid (SEQ ID NO:21) shown in Table 13A.

Table 13A. VIR11 Nucleotide Sequence (SEQ ID NO:21)

ATGAACGATGCTTCTCCCCGTCTGACCGAACGCGGCAGGCAACGCCGCCGCCCATGCTCGACGCCG
CTACCCAGGCCTTTCTCGAACACGGTTTCGAAGGCACACCCCTGGACATGGTGATAGAACGGGCGCG
TGGTTACGGGGGACCCTGTACAGCTCCTTCGGCGGCAAGGAGGGCCTGTTCGCCCGCGGTGATCGCC
CACATGATCGGGGAAATCTTCGACGACAGCGCCGATCAGCCGCGCCCCGCCACGCTGAGCGCCA
CCCTCGAGCATTTTCGGCCGGCGCTTTCTCACCAGCCTGCTCGATCCCCGCTGCCAGAGCCTCTATCG
CCTGGTGGTGGCGGAATCCCCGCGGTTTCGGCGGATCGGCAAGTCCTTCTACGAGCAGGGGCGCGAG
CAGAGCTATCTGCTGCTCAGCGAGCGACTGGCCGCGGTGCTCCTCAGATGGACGAGGAAACGCTCT
ACGCGGTGGCCTGCCAGTTTCTCGAGATGCTCAAGGCCGACCTGTTCCTCAAGGCCCTCAGCGTGGC
CGACTTCCAGCCGACCATGGCGCTGCTGGAAACCCGCCCTCAAGCTGTCCGTGGACATCATCGCCTGC
TACCTGGAACACCTGTGCGAGAGCCCCGCGCAGGGCTGA

The VIR11 protein (SEQ ID NO:22) encoded by SEQ ID NO:21 is presented using the one-letter amino acid code in Table 13B.

Table 13B. Encoded VIR11 protein sequence (SEQ ID NO:22)

MNDASPRLTERGRQRRRAMLDAATQAFLEHGFEGTTLDMVIERAGGSRGTYSSFGGKEGLFAAV
IAHMIGEIFDDSDQPRPAATLSATLEHFGRRFLTSLDPRCQSLYRLVVAESPRFFAIGKSFYE
QGPQQSYLLLSERLAAVAPHMDEETLYAVACQFLEMLKADLFLKALSVADFQPTMALLETRLKLS
VDIIACYLEHLSQSPAQG

5 MUT12

A *Pseudomonas* bacterial mutant (MUT12) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding PA0596. This gene encodes the VIR12 nucleic acid (SEQ ID NO:23) shown in Table 14A.

Table 14A. VIR12 Nucleotide Sequence (SEQ ID NO:23)

ATGTCTGATGATGCCCCGTTTCCAGCAGCTGAATTGCTGGTTGGACTCTTGTGTTGCCCGAGTTGTTCCG
TTGCCGAAGGTTGGGGGGAAGTGCCCCCGCCGAAC TGATCCCGGCCAGTAGCGACGCCAGCTTCCG
TCGTTATTTCCGCTGGCAGGGAGGGGACCGCAGCCTGGTGGTGATGGACGCGCCGCCGCCCCAGGAA
GACTGCCGACCGTTCTGTC AAGGTCGCCGAGCTGCTCGCCGAGCCGGCGTG CATGTGCCGAGGATTC
TCGCCCAGGACCTGGAGAACGGTTTCCTGCTGCTCAGTGACCTGGGCCGGCAGACCTACCTCGACGT
GCTTCATCCCGGGAATGCCGACGAGCTGTTTCAACCGGCCCTGGATGCGCTGATCGCCTTCCAGAAG
GTCGATGTCGCCCGGTGTCCTGCCCTGCTACGACGAAGCGGTGCTGCGCCGCGAGCTGCAGCTGTTCC
CCGACTGGTACCTGGCCCGCCACCTCGGCGTGGAGCTGGAGGGCGAGACGCTGGCCCGCTGGAAACG
GATCTGCGACCTGCTGGTACGACGCGCTGGAGCAACCGCGGGTGTTCTGTCATCGCGACTATATG
CCGCGCAATCTGATGCTCAGCGAGCCCAACCGGGCGTCCCTCGACTTCCAGGACGCCCTGCACGGCC
CGGTACCTACGATGTCACCTGCCCTGTACAAGGACGCCCTTCGTCAGTTGGCCCGGAGCCGCGCGTGCA
TGCCGCGCTGAACCGTTACTGGAAGAAGCGACCTGGGCCGGCATCCCGCTGCCGCCAAGCTTCGAA
GACTTCCTCCGTGCCAGCGACCTGATGGGCGTGACGCGCCACCTGAAGGTGATTGGCATCTTCGCC
GTATCTGTCACCGCGACGGCAAGCCGCGCTACCTGGGTGACGTGCCGCGCTTCTTCCGTTATCTGGA
AACCGCGTGGCGCGCGCTCCCGAGCTGGCCGAAC TGGCGAGCTGCTGGCCTCGCTGCCCGAGGGA
GCCGAGGCATGA

The VIR12 protein (SEQ ID NO:24) encoded by SEQ ID NO:23 is presented using the one-letter amino acid code in Table 14B.

Table 14B. Encoded VIR12 protein sequence (SEQ ID NO:24)

MSDDARFQQLNCWLDSCLELFAEGWGEVPPAELTPASSDASFRYFRWQGGDRSLVMDAPPP
QEDCRPFVKVAGLLAGAGVHVPRILAQDLENGFLLSGLGRQTYLDVLHPGNADELFEPAIDALI
AFQKVDVAGVLPAYDEAVLRRELQFDPWYLARHLGVELEGETLARWKRICDLLVRSALQPRVF
VHRDYMPRNMLSEPNPGVLDLFDALHGPVTYDVTCLYKDAFVSWPEPRVHAALNRYWKKATWAG

IPLPPSFEDFLRASDLMGVQRHLKVIGIFARICHRDGPRLGDPVPRFFRYLETAVARRPELAEL
GELLASLPQGAEA

MUT13

A *Pseudomonas* bacterial mutant (MUT13) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding PA5265. This gene encodes the VIR13 nucleic acid (SEQ ID NO:25) shown in Table 15A.

Table 15A. VIR13 Nucleotide Sequence (SEQ ID NO:25)

ATGAGCGGATTCCAGGACCAGAGTATCGACGAAGGCGTGCGCAAGCGCACCGCCTACCAGAACGATC
GGCGTGCACGACTGGCATTGAACGTCGAGCGACAGGACGGCGGTATCCTGCAGATTCCGGTGGCCAG
CGATATGCTCGGCCATGAGGAGCACGAGCGTATCCAGCAGAACACCTTCCTGGCTGTGATGCCGCTG
GTCCGCCTGCCAACGCTGGGCAAGGCCGGTTATGGCGACAGCTGCCCGCCGGCGCGCTACCGCGGG
CGGACGGATCTACCTGTTCCAGGACGGCAAGTTGTGGCGCGAACTGGAATGTGATGGCAAGGGCAA
CCTGTTTCAAGTCGATCTCCTGCAGGGGCGCAGCCAGCGTGCGGACAAGCGTCCGGCCTTAGGCAAG
ACACAAGCGCTGATCCTGGTGCCGGTGCTGGTCAAGGGGCAGTTCGTGATCCACGCTACACCATGG
CCTATAGCGAAACTCCCTGGCCTTGGTCTGATCATCGACTGGCTGGAGGAGGACCCGAGCGGGTCAA
CCGGCGCTGCCAGCAGATGGCGTCCGCTTGGAAACGCCTCGGTGGCCAACAGCACTGGAAAGCCTCC
ATCCATCAACCCGCGCTGGTCAATTGATCATCACGCCAGGGTTTGGCAGCTCGCGACTTCAACGTCG
AGAGCGCGCTGGAAGACCCGGCGGAATTCACACCTGAGTTTCGCCGCTTTTCGCGAAGAGTCGCTGGT
GTGCCAGTTGCAGCGACGCCAGCAGGAATTGGCGCCCTGCTGTAAGCAGGCTCCGCCCTCTGCGCTA
CCTACTCTGGAAGCCGGAGAGGACGTAAGTGAACCCCTCAAGCTGCGTGGCCATCCCAACCTCATCG
GGCTGATGCTCGACGACTCGCTGTTTCGCCTTGGCGCCAGCTGCGGCGCAGGCGCGCCACTGCGCCGC
CTACTTGGCGAGCCTCAATGCACTGCTGCCGCACCGTCCCAACGGACGCTATGCACAGGTGCTGAGC
AACATGCTCGACGGCCCGCTCGCCAAGCTCAGGGGCGAGGTTCGATCAGGCCGAACCTGGACGAGGCCA
TCTTCGCCGAGGAGCGACAGTCTTGGCGAATCCACCTGACGCGAGCAGGTTCGAGCATCTGGTTGCCCT
GCTGGAAGGCCCTTGCACCCGGTGTTCGAGGACTGGACCCACAGTGCAGCAAGCCCTGCTGGAG
CCCTACAGCCTGATGAGCGAGGCACTGGCTGCGCTGAACAGCTTCCCGACCGCTGCGACGCACTGT
ACAGCGGTACCGCCTACCGGGCGCTGGCGGCACATGTCGAGCGGGTGGTTCAGCAGGTTCTGCAGGC
AAGCCACCCGCTTGGCGCCATGCTCCTGGCCAAGGACGAAGGACAACCTCCCGAGCCGTTTCGGCGC
CTGCAGGCGCTGCGCGATAGCCCGCGGACGCCGACCCGATGCAATGGGCTTCAGCAGCTGATGC
TGGGAGCCAGTCTGCTGGGCGAGGTTCAGCAGCCAGCGCCGGAAGAGCCTCGCCTACTTCCTCGG
CGACCTGCTGGACGTGTTTCGGCGCCAGCGTAGTCGAGCAACTCGGCCGGCTGTCCAGGGCGCCACC
CAGATCCAGCTCGACCGCTTGTTCGCACCGACCTTCAATACCTGAGCGCCCTCTCGGTGAAGATGA
AAGGTATCCGCCCTGCTGCCCGACAGTCAGGTGCCGCTCGACATGGTTGTCGTCGGCTGCGCGGAGC
CGGCCTGCGCAACGGTCTGACCGAGGTTCGAGCGCCAGGAGCTGAGGCGCAAGAGCTATCGGCGCGCC
ATCGTTCAGGACGGTGC CGGCAATCCCTGGCCGGCACCAGTCCCGCGACACCGGCATGAGTCGCG
CCAACCTGCGCAACGTCATGGTGGTGGCGGTACCCAAGGATCACCCGGACCTGCTTGCCCTACACGAA
ATTCCGTACGCACTTAGGCACGTTGACCCAGGTGATGGAGAACAACCTCGCATCGTGCCGACGATGATG
CTGGGGTTTGGCGATTATAACTTGAATGTGCAAGTGCAGGCATACAGTGGCTTTGTAGACAGTGGAG
AAAAGCACAGAGGACGATCGGGGCTGTGCGGTGCAGTAATCGATTTAACAGCCGCTGGAGGAAGCCA
TGCAAAGCTGCTTTTCGGACCATCTACTGCAAAGTATCTAGAAACCCACGATATATCGGTAGCCCAA
ATATCCCTTCGATGGGCCAGGAATCTAGAAGTTCAAACAGGCAGCCCTAAGTTAGGGTTGCTACGTG
GGCTTGGTGGCGCAGCCACACTATTCGGTGCAGGCATCAGTGTATGGGATGGCTACCGAGCTTTGAG
GCAGGGAGATAGCGATGCGGCTGCGGCCCTACGTTGTGGCCGAGTGGGTGGGGGCTTTGGGGTGCC
TACGTCTAGGATGGATAGTAAACCTTATGCTTTGCTGGCTGGTGGCTTTTGGCGATCGGAGGCA
CTGTGGTTCGCTAATCTACTGACTGACAGCGATGCGGAAACCATCGTAAAGAAAGGCCCTTCGGCCG
GCAATTCGCGGAGGCTGGCCTGCTCGATTTCGCTGATGGGCCAGGACAGCGCTTCGCCCATCTGAAA
GACCCGCAACGGCCTATCGCCAATTGCTGGGAGTCTTCGGCCATCCGCGGGTCTTTGTCCATCGCC
TGGAAGACTGGCGCAAAATTGGCGCCGGCGGCGCATCGATCTGTCTTCAGGAAGCGGAACGGGGTTCG
CCAAGCGGTTCAGCCGCACTGCGCTATCCTGCATCGACCCCAAGTTGCAGGCGCTGGAGGCAACGAT


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TGGGCCGTGGTGCTGAGTTCCCCGCTCCTGGCCATGTTTCGAGAATGGCCAGAAGGCGTTCCGCCTGG
TGGCCCAGGAGTTTCTCAGCAGCTTGGCGATCGATCCGGGACCCCTGTTTCGGCGTCAAGCGCTACCA
TCGGGTCCCCCGGGGGCCCCGCAAGCTCGAAGCCTTGGCGTTGGATGCTGCCAGCGTGCTCTATGTG
CTGCCGGCCAGCCTGCCGATTCCGCAGTTGTCTCCTCGGGCCCGCTATAGCATGCGCATGACCCAGG
GTTTGAAGATCAGCGCACAGTTTCAACTCAATGCCGACCAGCCTGAGCAGCGGCTTGTCTGCCTCA
ACCCAGCCCCGAAGAGTTGGAGTGCATTACATCCGCCAATCGGTACCTTCCCCCGGACGACTTGGGC
CCCCATGCTGCGCCACCTTATTGGTTGATAGAGAACAGTGAGTTCAACGTATGA

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The VIR13 protein (SEQ ID NO:26) encoded by SEQ ID NO:25 is presented using the one-letter amino acid code in Table 15B.

Table 15B. Encoded VIR13 protein sequence (SEQ ID NO:26)

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MSGFQDQSIDEGVRKRTAYQNDRRARLALNVERQDGGILQIPVASDMLGHEEHERIQQNTFLAVM
PLVRLPTLGKAGYGDQLPAGALPRAGRIYLFQDGKLWRELECDGKGNLFEVDLLQGRSQRADKRP
ALGKTQALILVPLVKGQFVIPRYTMAYSETFPWPWSYIDWLEEDPQRVNRRCCQMASAWNANVAN
QHWKASIHQPALVIDHHAQGLRPRDFNVESALEDPAEFTPEFAAFREESLVCQLQRRQQLAPLL
KQAPPSALPTLEAGEDVLETLKLRGHPNLIIGLMLDDSLFALRHAAAQARHCAAYLRSLNALLPHR
PNGRYAQVLSNMLDGLAKLRGEVDQAELEDAIFAEERQSCRHLTQQVEHLVALLEGPLHPVLQ
DWITHQCDEALLEPYSLMSEALAALNQLPDRCDALYSGTAYRALAAHVERVVSTVLQASHPLGAML
LAKDEGQLPEFVRRLQALRDSPTPDAMDGLSTLMLGASLLGEVDQPSAGKSLAYFLGDLLDVF
GASVVEQLGRLSQGATQIQDLRDLFAPTFNTLSALSVKMKGIRLLPDSQVPLDMVVVGVRGAGLRN
GLTEVERQELRRKSYRRRAIVQDGAGNPLAGTSPRDTGMSRANLRNVMVAVPKDHPDLLAYTKFR
TQLGTLTQVMENRIVPTMMLGFAIYNLNVQVQAYSQFVDSGEKHRGTIGAVGAVIDLTAAGGSH
AKLLFGPSTAKYLETPRISVAQISPRWARNLEVQTSQPKLGLLRGLGGAATLFGAGISVWDGYRA
LRQGDSDAAAAYGVAAVGGGLWGAYVLGWIVNPNYALLAGAVLAIGGTVVANLLTSDAETIVKKG
PFGRQFAEAGLLDSLMGQDQRFALHKDPQTAYRQLLGVLGHPRVFVHRLEDWRKLAPAAHRSVLQ
EAERGRQAVSRTALSCIDPKLQALEANDWAVVLSPLLAMFENGQKAFRLVAQEFLLSLPIDPGT
LFGVKRYHRVPAGPAKLEALPLDAASVLYVLPASLPPIQLSPRARYSMRMTQGLKISAQFELNAD
QPEQRLVLPQPSPKSWSAFTSANRYLPDDDLGPHAAPPYWLIENSEFNV

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5

MUT14

A *Pseudomonas* bacterial mutant (MUT14) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding pyochelin biosynthetic protein pchC (PA4229). This gene encodes the VIR14 nucleic acid (SEQ ID NO:27) shown in Table 16A.

Table 16A. VIR14 Nucleotide Sequence (SEQ ID NO:27)

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ATGAGCGCCGCTGGGTCCGGCCGTTCCGCCTGACGCCGATGCCGCGCCTGCGCCTGGCCTGCTTCC
CCCATGACAGGCGGCAGCGCCAGCTTCTTCCGTAGCTGGAGCGAACGCCCTGCCGCCAGACATCGACCT
GCTTGCCCTGCAGTACCCGGGTGCGGAGGACCGCTTCAACGAGGCGCCGCCACCCGCTGGAGGAC
CTCGCCGACGGCGCCGCCCTCGCCCTGCGCGATTTCGCCGACGCGCCCTGGCGCTGTTCCGCCACA
GTCTCGGCGCGGCGCTGGCCTACGAAACCGCCCTGCGCCTGGAAAGCGCCGGCGCGCCGCTGCGCCA
CCTGTTCTCTCCGCCATCCGGCACCGCACCGGCAACGCGGCGGCGCGTTGCACCGCGGCGACGAG
GCGGCGCTGCTGGAGGACGTCCGCCGCCAGGTTGGCGCCAGCGAGCTACTCGAGGACGCCGACCTGC
GCGCGCTGTTCTTCCGATCCTGCGCGCCGACTACCAGCGGATCGAGACCTACCGACGGGCGCAGCC

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CATCGCCCTGGCCTGCGCCCTCGACGTCCTCCTCGGCGAGCACGACGAGGAAGTCAGCGCCGCCGAG GCGCAGGCCTGGAGCGACGCCAGCCGACTCCCGCCAGGCTGCGGCGCTTTCTTGGCGGCCACTTCT ACCTGAGCGAGGGGCGCGACGCGGTGATCGAGCACCTGCTGCGCCGCTCGCACATCCCGACGCCCT TTCCCGAGAGGTTGCATGA
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The VIR14 protein (SEQ ID NO:28) encoded by SEQ ID NO:27 is presented using the one-letter amino acid code in Table 16B.

Table 16B. Encoded VIR14 protein sequence (SEQ ID NO:28)

MSAAWVRPFRLTPMPRLRLACFPHAGGSASFRRSWSERLPPDIDLLALQYPGREDRFNEAPATRLLEDL ADGAALALRDFADAPLALFGHSLGAALAYETALRLESAGAPLRHLFVSAHPAPHRQGGALHRGDEAA LLEDVRRQGGASELLEDADLRALFLPILRADYQAIETYRRAQPIALACALDVLLGEHDEEVSAEEAQA WSDASRTPARLRRFPGGHFYLSGRDAVIEHLLRRLAHPDALSREVA
--

MUT15

A *Pseudomonas* bacterial mutant (MUT15) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding dihydroaeruginic acid synthetase pchE (PA4226). This gene encodes the VIR15 nucleic acid (SEQ ID NO:29) shown in Table 17A.

Table 17A. VIR15 Nucleotide Sequence (SEQ ID NO:29)

ATGGATCTGCCCCCGATTCCCGTACCGCCCTGCGCGACTGGCTGACCGAGCAGCTCGCCGACCTGC TCGGCGAACCGCTTGCTGACGTGCGCGCCCTGGCGGACGACGACGACCTGCTGGGCTGCGGCCCTCGA CTCGATCCGCCCTGATGTACCTGCAGGAACGCCCTGCGCGCGCGTGGCTCGACGCTGGACTTCGCCCCAG TTGGCGCAGCGCCCTGCTTGGGGGCTGGCTGACCTGCTGGGCTGCGCGGACCGGCTGTCCGCC CGGCAACGGTTCGCGCTGCCGACGGCGCAGGATCGCGATCAGCCGTTTCGAGCTGTCTCCGTGTCAGCA GGCTACTGGCTGGGACGTGGCGCCGGCGAGGTGCTGGGCAACGTCAGCTGCCATGCCCTTCTGGAA TTCCCGCACGCGGGATGTCGACCCGCGAGCGCCTGGCCGCGCGCGGCGAGTGGCTGCGTCAACGCCACC CGATGTTGCGGGCGCGCTTCCTCGACGGTGCACGAGATCCTTCCGACGCCGCCGCTGTCTGCTT CGACCTGCAGGACTGGCGCACCTTACAGGTGGACGAGGCCGAGCGGACTGGCAGGCGCTGCGCGAC TGGCGCGCCCATGAATGCCCTGGCGGTGGAGCGCGGCCAGGTGTTCTGCTCGGGCTGGTGCAGCATGC CGGGCGCGGAGGATCGCCTCTGGCTGAGTCTCGACCTGCTTGGCGCCGATGTCGAAAGCCTGCGCCT GCTGCTGGCCGAACTGGGCGTTGCCCTACCTGGCGCCGAGCGCCTGGCGGAGCCGCCGCGCTGCAT TTCGCCGACTACCTGGCGCACCGTGGCGCGCAACGCGCCGAGGCCGCGCGCGCGGCGGCTGACT GGCTGGAAACGCTGCCGCGCTTGGCGGACGCGCGCCCTGCCGTTGGCTGCGCGCCGGAAAGCAT CCGCCAGCCGCGCACCCGGCGCCTGGCATTCAGCTTTCCGCCGCGAGAGCCGCGCTGGAGCGT CTTGCCGCGCAGCATGGCGTGACCTTGTCCAGCGTGTTCGGCTGCGCCTTCGCGCTGGTCTGGCGC GCTGGAGCGAAAGCGCGGAATTTCTCCTCAACGTGCCGTTGTTTCGATCGGCATGCCGACGACCCGCG TATCGGCGAGGTGATCGCCGACTTCACCACCTGTGTGCTGCTGGAGTGCCGGATGCAGGCCGGGGTG TCCTTCGCCGAGGCGGTGAAGAGCTTCCAGCGCAACCTCCACGAGCCATCGACCACGCCGATTC CCGCCCTGGAGGTGCTCCGCGAGGCGCGCGGCGGCGAGCCAGCCAGCTCGGCGCGCGTGGTGTTCGC CAGCAACCTGGGCGAGGAGGGCTTCGTCCCGCGCGCCTTCGCGGACGCTTTCGGCGATCTCCAGAC ATGCTCTCGCAGACCCCGCAGGTCTGGCTCGACACACAGCTCTACCGGGTGGGCGACGGTATCCTGC TGGCCTGGGATAGCGTCTGCGCCTGTTCGCCGAAGGTCTGCCGGAACCATGTTGGAAGCCTACGT GGGCTGCTCCAGCGTCTTGCAGACGCGCTGGGGGCGACCCGCCGATCTGCCGTTGCCCTGGGCG

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CAGCAGGCGCGCCGGGCCCTGCTCAACGGCCAGCCGGCATGCGCCACGGCGCGCACCTTGCATCGCG
ACTTCTTCCCTTCGCGCCGCGGAGCGCCGGATGCCGACGCGCTGCTCTATCGCGACCAACGTGTAC
CCGCGGCGAACTGGCCGAGCGTGCCTGCGCATCGCCGGCGGCTTCGCGGAAGCCGGGGTGCGCCCT
GGCGACGCGGTCGAGGTACGCTGCCGCGCGGACCGCAGCAGGTGCGGGCGGTATTCGGCGTGTCTCG
CCGCGAGGCGCTGCTACGTGCCGCTGGACATCGACCAGCCGCCCGCACGGCGGCGCTGATCGAAGA
GGCGCGCGGGTATGCCTGGCGATCACCGAGGAGGACGATCCGCGAGGCCTTGCCGCGCGCTGGAT
GTCCAGCGCCTGCTGCGGGCCCCGGCGCTGGCCGCCCGCTGCGCGCGCGAGGCGAGTGCCT
ATGTGATCTACACCTCGGGCTCCACCGGGGTGCCAAGGGCGTCGAGGTACGCCACGGCGGCGGAT
CAATACCATCGACGCGCTGCTCGACCTGCTGCGGGTGAACGCATCGGATCGCTTGTGGCGGTCTCC
GCGCTGGACTTCGATCTGTGGTCTTCGACCTGTTGCGCGGCTCGCGCGGCTGCCAGCCTGGTCC
TGCCGGCCAGGAACAGGCGCGCGATGCCGCTGCTGGGCGGAGGCTATCCAGCGGCATGCGGTGAG
CCTGTGGAACCTGGCGCGCGGCTTGTGAGATGGCCCTCAGCCTGCCGGCGAGCCAGGCCGACTAT
CGCAGTCTGCGGGCGGTGCTGCTGTCGGCGACTGGGTGGCCCTGAGCCTGCCGGCGAGCCAGGCCGACTAT
CACGTTGTGCCGAAGGCTGCCGCTGCTGCTGGGCGGCTGAGCCTGCCGGCGCGCTGCGCC
CCTGCAGAGCGTCGATACGGTGGCGCGCACTGGCGTTCGATTCCCTACGGCGGCGCATGCGGTGAA
CAGGCCTACCGGGTGGTTCGACACCCACGGGCGCGACGTGCCGGACCTGGTGGTGGCGAGCTGTGGA
TCGGCGGCGCCAGCCTGGCGCGCGGCTATCGCAACGATCCCGAAGTACAGCGCCCGCGCTTTCGTCCA
CGATGCCAGGGCGGCTGGTATCGCACCGGCGATCGCGGTCGCGGCGAGCGCATCGAGTTGGGCGAGGTGGAG
TTCCTCGGTGGGTTCGACAGCAGGTGAAAGTGCAGCGGCGCGGCGCGCATCGAGTTGGGCGAGGTGGAG
CCGCGCTGTGCGCCAGGCTGGCGTGGAGAGCGCTGCGCGCGGCGGTGCTCGCGCGTGGCGTGGCGAG
CCTCGGCGCGGTGCTGGTACCGCGCCTGGCGCCACGGGCGCAAGGCTCCATGGATCTACCGGCGCA
CAGCCCTTCGCGCGGCTGGCAGAGGCGAGGCGGTACTACCGGGGAAATCCTCGGCGCGCTGCTGG
AGGCGCGGCTGGAGCTAGACGACGGTTTGCAGCGGCGCTGGCTGGACTGGCTAGCGGACTCCGCGCG
CAGCGCGCTGCCGTCGCTCGACGAGGCGTTGCGCGGCGCTGGCTGGACTGGCTAGCGGACTCCGCGCG
ATGGGCAACGCTCTGCGCGGCTGCTCGCGGCGCAACAGGCGCGCGGCGCGCTGCTCCTCGATCCCT
GGCTGGCGCGCGAGGCGGTGGCGCGCGCCTGCCGGACGGCGCGAGGCGCTGGCGCGCTGCTCGA
AGCGCTGCCGACGCGGCTGCCGCGCAACGCTGCGGGTGGCGGTGCTGGATACCGCGCGCGGCTC
TGGCTCGACACGAGGCTAGGCTCGCTGTTGCGCCAGGGCTGGAAGTACCCCTCTTCGAACGAGCC
GCGTCTCTCTCGACGCGCGCGCCACCGCTTGCGCGGAACGGATCGTGGTGCAGGCGCTGGACGACGG
CCTGCTACCTGCCGAGCACCTCGGTGCTTACGACCGGGTGATCAGCTTCGCGCGCTGCACGCCTAC
GAGGCCAGCGCGAAGGCTGGCGCTGGCGGCGCGCTGCTGCGCGCGCAGGGCGCGCTGTGTGCTGG
TGGACCTGCTATGCGAGTCGCCACTGGCGCTGCTCGGTGCGGCTTGTCTGACGACCGCGCGCTGCG
CCTGGCGGAGCTGCCGAGCCTGTTGGCGGATCTCGCGCTGCGGGACTGGCGCGCGCTGCTGTGG
CGCAGCGAGCGGATCGCCCTGGTCGAGGCGCTGGCACCGGACTCGGGCTCGACGCGCGCGCGCTCC
AGGCCGCGCTGGAGCAACGCTGCCCGAGGCGATGCGGCGCGCAACGCTGTGGTGGCTGCCAAGCCT
GCCGTTGAACGCAATGGCAAGGTCGATCGTCGCGGCTGGCGGAGAGCATGACCCGCGCACTCGGC
GAGTGTGCTACGAGCCCTCGGCGGAGGAGCGCTGGAAGCCATGAGCAAGCGCTGGCCGAGTGCT
GGGAAGCGGTTCTCAAACGCGCGGTGCGTCTGCGGAGGCGAGCTTCTTACAGCTCGGCGGCGACAG
CCTGCTGGCGACCGCGCTGCTGGCGGCGATACGTGAGCGTTTCGGCGTACGCTGGGCGATGGCCGAC
TTCTATCGCCAGCCGACCTGGCCGGTCTTGCGCGCACTTGACAGGTGCAGACCGTCGAAATCGAGG
AAACCAACTGGAAGAGGCGGTGCTATGA

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The VIR15 protein (SEQ ID NO:30) encoded by SEQ ID NO:29 is presented using the one-letter amino acid code in Table 17B.

Table 17B. Encoded VIR15 protein sequence (SEQ ID NO:30)

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MDLPDPSRTALRDWLTEQLADLLGEPLADVRLADDDLLGCLDSIRLMYLQERLRARGSTLDFAQL
AQRPCLGAWLDLLACADRLSAPATVALPTAQDRDQPFELSSVQQAYWLGRGAGEVLGNVSCHAFLEFR
TRDVPDQRLAAAECEVRQRHPLRARFLDGRQQILPTPLSCFDLQDWRTLQVDEAERDQALRDWRA
HECLAVERGVFLLGLVRMPGGEDRLWLSLDLLAADVESLRLLLAELGVAYLAPERLAEPALHFADY
LAHRAAQRAEAAARARDYWLERLPRLPDAPALPLACAPESIRQPRTRRLAFQLSAGESRRLERLAAQH
GVTLSVFGCAFALVLARWSESAEFLNVLPLFDRHADDPRIGEVIADFTTLLLECRMQAGVSFAEAV
KSFQRLHGAIDHAAFPALEVLREARRQGQPRAPVVFASNLGEEGFVPAAFRDAFGDLHMDLSQTPQ
VWLDHQLYRVGDGILLAWDSVVGLFPEGLPETMFEAYVGLLQRLCDSAWGQPADLPLPWAQQARRALL
NGQPACATARTLHRDFFLRAAEAPDADALLYRDQVRVTRGELAERLRIAGGLREAGVRPGDAVEVSLP
RGPQQVAAVFGVLAAGACYVPLDIDQPPARRRLIEEAAGVCLAITTEEDDPQALPPRLDVQRLLRGPAL
AAPVFLAPQASAYVIYTSGSTGVPKGVEVSHAAINTIDALLDLRVNASDRLLAVSALDFDLSVFDL
FGGLGAGASLVLPAQEQARDAAWAEAQRHAVSLWNSAPALLEMALSLPASQADYRSLRAVLLSGDW
VALDLPGRRLRPRCAEGCRLHVLGGATEAGIWSNLQSVDTVPPHWRISIPYGRPLPGQAYRVVDTHGRDV

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PDLVVGELWIGGASLARGYRNDPELSARRFVHDAQGRWYRTGDRGRYWDGDTLEFLGRVDQQVKVRGQ
RIELGEVEAALCAQAGVESACA AVLGGGVASLGAVLVPR LAPRAEGSMDLPAAQPFAGLAEAEAVLTR
EILGALLEAPLELDDGLRRRWLDWLADSAASALPSLDEALRRRLGWQAAGLTAMGNALRGLLAGEQAPA
ALLLDPWLAPQAVAAARLPDGREALARLLEALPTPAAGERLRVAVLDTRAGLWLDQGMASLLRPGLLELT
LFERSRVLLDAAATRLPERIVVQALDDGLLPAEHLGRYDRVISFAALHAYEASREGLALAAALLRPQG
RLLLVDLLCESPLALLGAALLDDRPLRLAELPSLLADLAAAGLAPRCLWRSERIALVEALAPGLGLDA
AALQAGLEQRLPQAMRPERLWCLPSLPLNGNGKVDRRRLAESMTRALGECRHEPSAEEPLEAHEQALA
ECWEAVLKRFPVRRREASFFSLGGDSL LATRLLAGIRERFVGRLGMADFYRQPTLAGLARHLQVQTVET
EETQLEEGVL

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MUT16

A *Pseudomonas* bacterial mutant (MUT16) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding pyochelin synthetase *pchF* (PA4225). This gene encodes the VIR16 nucleic acid (SEQ ID NO:31) shown in Table 18A.

Table 18A. VIR16 Nucleotide Sequence (SEQ ID NO:31)

```

ATGAGCCTCGGCGAAGCTGCTGGAACCTGCCGAGCCGGCGCATCGAAGCTCTGGAGCGAGGCGGGCC
GCCTGCGCTATCGCGCCCCCAGGGTGCCCTCGACGCGCGCCTCGCCGAGCGCCTGCGGGCCGAGCG
CGAGGCCCTGCTGGAACACCTGGAAGCGGCCCTGGCTGGCGCGCCGAACCCGACATGGCCCCACCG
CGCTTCCCGCTGACCCCGGTGCAGGCGCCTACGTGCTGGGCGCGCCAGGCGGCCCTTCGACTACGCG
GTAACGCCTGCCAGCTGTACGCCGAGTACGACTGGCCGGCCGACACCGATCCGGCGCGCCTGGAGGC
GGCCTGGAACGCCATGGTCGAGCGCCACCCGATGCTGCGCGCGGTGATCGAGGACAACGCCTGGCAG
CGCGTGCTGCCCGAGGTGCCCTGGCAGCGGCTGACCGTGCATGCCTGCGCGGGGCTCGACGAGGCCG
CTTTCCAGGCGCACCTGGAGCGGGTCCGCGAACGCCCTCGACCACGCCTGCGCGGGCGCTCGACGAGT
GCCGGTCTGCGCCCCGAGCTGAGTATCGGCCGGGATGCCTGCGTACTGCACTGCTCGGTGGATTTC
ACCCTGGTCGACTACGCCAGCCTGCAATTGCTGCTTGGCGAATGGCGCCGCGCTATCTCGATCCGC
AATGGACGGCGGAACCGCTGGAGGCGACCTTCCGCGACTATGTGCGCGTCGAGCAGCGCCGACGCCA
GTCGCCAGCCTGGCAGCGCGACCGGACTGGTGGCTGGCGCGTCTCGACGCGCTACCGGGGCGTCCC
GACCTGCCGCTGCCGGTGCAGCCGGACACCCGGTCCACGCGCTTCCGGCACTTCCACGCGCGCCTCG
ACGAGGCGCGCTGGCAGGCGCTCGGCGCGCGCGCGCGCGGAACACGGCCTGAGCGCTGCCGGCTGGC
CTTGGCGGCTTCCGCCGAGACCATCGGTGCTGGAGCCAGGACCGGCGTTCTGTCTCAACCTGACG
GTACTCAACGGCGCGCGCTGCATCCGCAGCTGGCGCAGGTGCTCGGTGACTTCACCGCGCTCAGCC
TGCTGGCAGTGGACAGCCGCCACGGCGACAGTTTCGTGAGCGTGCCCGACGCATCGGCGAGCAGAT
GTTTCGACGACCTCGACCAACCGACCTTCAGCGCGCTCGACCTGCTGCGCGAAGTGGCGCGCGCGGT
GGTCGCGCGCGCGATCTGATGCCGGTGGTGTTCACAGTGGCATCGGCAGCGTGCAGCGCTGCTCG
GCGATGGCGAGGCGCGCGCGCGCCACGCTACATGATCAGCCAGACCCCGCAGGTCTGGCTGGACTG
CCAGGTACCCGACAGTTCCGGCGGCCCTGGAGATCGGCTGGGACGTACGCCTCGGGTTGTTCCCCGAG
GGCCAGGCGGAAGCCATGTTCCAGCACTTCGTCCGGCTGCTCCGGCGCCTGGCGCAGAGCCCGCGCG
CCTGGACCGGACCGCGATGCCACGGAACCCGTCGAGGCGCGCGCGCAGGCGTTGCCCGGTAGTGCCCG
GAGCATCGCCCGCGGTTTCGCCGAGCGTGCCCTGCTGACCCCGGACGCCACGGCGATCCACGATGCC
GCCGGCAGCTACAGCTACCGCCAGGTGCGCCAGCACGCCAGCGCCCTGCGCCGCGCTCTGGAAGCGC
ACGGCGCGGCGCGTGCCCGGCGGGTCCGCGGTGATGCTGCCGAAAAGCGCGCGCAATTGGTCGCGGT
GATCGGCATCTCCAGGCGCGCGCGCCTATGTGCCGGTGGACATCCGCCAGCCTCCGCTGCGGCGC
CAGGCGATCTCGCCAGCGCGCAAGTGGTCGCGCTGGTCTGCTGGAAAGCGATGTCCCGGACGTCG
GCTGCGCCTGCGTGGCCATCGACCGGTGGCGCGCGGACAGCGCCTGGCCGCCACCGCCGCGGGA
GGTGGCGGCGGACGACCTCGCCTACGTGATCTACACCTCCGGCTCCACCGGCACGCCAAGGGCGTG
ATGCTCAGCCATGCGGCGGTGAGCAACACGCTGCTCGACATCAACCAGCGCTACGGCGTCGACGCCA
ACGACCGGCTCTCGGCTCGCCGAGCTGAGCTTCGACCTCTCGGTCTACGACTTCTTCGGCGCCAC
CGCGCGGGGGCCAGGTGCTCTCCCGGACCCGGCGCGCGGCGGCGGATCCATCGCACTGGGCGGAA
CTGCTGGAACGCCACGCCATCACCTGTGGAACCTCGGTGCCGCGCCAAAGGCCAGATGCTCATCGATT
ACCTGGAGAGCGAGCCGCAACGTCACCTGCCGGGACCGCGCTGCGTGTCTGGTCCGGTACGTTGGAT

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TCCGGTCAGCCTGCCGACCCGCTGGTGGCGGCGCTGGCCGGACAGCGCGCTGTTTCAGCCTGGGCGGC
GCCACCGAGGCGGCGATCTGGTCGATCGAGCAGCCGATCCGCCCGCAGCACACCGAGCTGGCCAGCA
TCCCTTATGGCCGTGCCCTGCGCGGGCAGAGCGTGGAAGTCTTGGATGCCCGCGGGCGGCGCTGCC
GCCGGGCGTGCGCGGCGAGATCCATATCGGCGGGGTGGGCTGGCGCTCGGCTACGCCGGCGATCCG
CAGCGCACCGCCGAACGCTTCGTCCGTACCCCGATGGCCGTGCGCTGTATCGCACCGGCGACCTCG
GCCGCTACCTGGCCGACGGCAGCATCGAGTTCTCGGCCGCGAGGACGACCAGGTGAAGATTGCGGG
CCACCGCATCGAACTGGCCGAACCTGGACGCCGCGCTGTGCGCTCATCCGCAGGTCAACCTGGCGGGC
ACCGTGGTGTCTCGGCGAGACCCACGAGCGCAGCCTGGCCAGCTTCGTACCCCTGCATGCGCCGGTGG
AGGTGGCGAGGATCCGCGTACGGCGCTCGACGCGGTGCGCCAGCGGGCGGCCAGGCCCTTGCGCCG
CGACTGGGGCAGCGAGGAGGGCATCGCCGCGGCGGTGGCCGCACTCGACCGTGCCTGCCTCGCTCG
TTGGCCGCTGGCTGGCCGGCAGCGGTCTGTTCCGCACTGCGACGCGCTGGACTTAGCCACCTGT
GCCAGCGCCTGGGTATCGCCGAGGCGCGCCAGCGCCTGCTGCGCCACTGGTTGCGCCAACCTGGAGGA
GGGCGGCTACCTGCGCGCCGAGGGCGAGGGCTGGCTGGGCTGCGCCGAGCGTCCCGCGCAGAGTCCG
GAGGACGCTGGACGGCGTTTCGCCGGCTGCGCGCCGCGCGCTCTGGCCGGCCGAGCTGGTTCGCT
ACCTGCGTGACAGCGCGCAATCCCTCGGCGAGCAACTGGCCGGGCGGATCAGCCCGCGCGCTGAT
GTTCCCGCAGGGCTCGGCGCGCATCGCCGAGGCCATGTACAGCCAGGGCTGCATGCCAGGCGCTG
CACGAGGCCATGGCCGAGGCCATCGCCGCCATCGTCGAGCGCCAGCCGCAACGGCGCTGGCGCCTGC
TGGAGCTTGGCGCGGCGACCGCCGCCCGCCAGCCGACGGTGATCGCCCGGTTGGCGCCGCTGGTGCA
CGAGGGGCGGAGGTGGACTACCTGTTTACCGACGTTTCCAGCTACTTCTCGCCGCGCGCCGCGAG
CGCTTCGCGGACCGAGCCGTGGGTACGCTTCGCCGCTTCGACATGAACGGCGATCTTCTCGACCAAG
GCGTGGCGCGCGCACTCGGTGGATATCCTGCTCAGCTCCGGGGCTTGAACAACGCGCTGGACACCC
GGCGCTGCTGGCCGGCTGCGCGAGTTGCTGAGCGCCGACGCTGGCTGGTGATCCAGGAACCTGACG
CGCGAGCACAACGAGATCAGCGTCAGCCAGAGCCTGATGATGGAACCCGCGCGACCTCCGCGACG
AGCGCCGCAACTGTTCTCCACACCGGCAATGGCTGGAGTGGCTGGCGGCACAGGGTGGCGACCT
GGCTTGTGGGGTGGTGCCGCGGGCAGCGCTCTCGACCTGCTTGGCTACGATGTCTGCTGGCTCGC
TGCAAGACCGACCGCGCCCGCTGGAGCCGGCCGAGCTGCTGGCTTTCGTCGAAGCGCGGGTGGCG
GCTACATGCTCCCGGCGCAGTTGCGCGTGTCTGAACGCTTCCGGTACCGGCAACGGCAAGATCGA
CCGCAAGGCCCTGACCGGCTTTCGCCCGCCAGCCCCAGGCGGACCTTCGGCATGGCGTCGCGCAGGCA
CCGCGCGACGAACCTGGAGAATGCGCTGCTGGCACTCTGGCGGGAGGTGCTGGACAACCCGTGCTGG
CGCTCGAGCAAGACTTCTTCGGGGCCGCGCGGCACTCGCTGTTGATCGCCAGTTGATCGCCCGTTT
GCGCGAACGACTGGAAGCGCCCGTTCGGCATCCGTTTCGATCGCCTGCTACGCTGGCGCTCAGCCAG
CCGACGCGCGCGCGGCTGGCCGAACGCTGCGCAGCGCGCCGGAAGAGGGCGTGGGCCAGCCCTGG
CCGCGGCGCGCGGCTGCGCCCGCGCGCGCGCGGCGATGTCGCGCGCACCGCTCGCCGAGGGCGCGGT
GGCGCTCGACCCGCTGGTGCGCTGGTGGCCGGCGAGGGCGTGGCGGGGTGCTGGTCCACGAAGGC
CTCGGCACGCTACTGCCGTACCGCCCGCTGCTTCGCGCCCTGGGTGAGGGGCGGCGCTGCTGGGGC
TGGCCGTGCATGACAGCGACGCTACCTGGCGATCCCGCCGAGCATCTCAACGCTGCCTCGGCCG
CCGCTACGCGGAGGCGTCCATCGCGCGGGCTACGCGAGGTGACCTGCTCGGCTACTGCTCCGGC
GGGCTGGTTCGCCCTGGAGACCGCCAAGTCCCTGGTCCAGCGCGGGGTGCGCGTGCGCCAATGGATA
TCGTTCCAGCTACCGGATTCCCTACCGGGTGGACGACGAGCGCTGCTGTTGTTTACGCTTCGCCG
GACCTTCGGCTGGATACCGCGGCGCTCGGCTTCGCCGCGCGGAACGCTTCGCCAGGCGGTGCAG
GCGGCGCTCGCGCAGACACCGGAGCGCTGGTTCGCCGAGGCGCTGGCGGGGTGCGCGGCTGGCCG
ATCTCGTCGCCCTGCGCGGCGCGCTGCTACAGGCGGCCAGCGGTAGCGCCGACGCGCTCAGCGTCGA
ACGCGACACCTCTACCGGCTGTTCTGTCTACTCGGTGCGTGCCAGCCAGGCCGAGGCGCGGAGCCC
TACGTGCGCGCGCTGCGGCTGTTCTGTGCTACTCGGTGCGTGCCAGCCAGGCCGAGGCGCGGAGCCC
CTCTGGAGACCAATGCGCGGCGCGCGCGCTTGGCGCGTGGCGCATCCACGAGGTGCCCGGCGGCA
CTTCGACTGCCTGGGCGAAGCCCTGGCGCAATCTTGTGCAACCCATGCCAGAGGAGGCGAGCCGA
TGA

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The VIR16 protein (SEQ ID NO:32) encoded by SEQ ID NO:31 is presented using the one-letter amino acid code in Table 18B.

Table 18B. Encoded VIR16 protein sequence (SEQ ID NO:32)

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MSLGELLETCRSRRIELWSEAGRLRYRAPQGALDAGLAERLRAEREALHLEGGPGWRAEPDMA
HQRFPPLTPVQAYVLGRQAADFYGGNACQLYAEYDWPADTDPARLEAAWNAMVERHPLRAVIED
NAWQRVLPPEVPWQRLTVHACAGLDEAAFQAHLEVRERLDHACAALDQWPVLRPELSIGRDACVL
HCSVDFTLVLDYASLQLLLGEWRRRYLDPQWTAEPLEATFRDYVGVEQRRRQSPAQRDRDWWLAR
LDALPGRPDLPLRVQPDTRSTRFRHFHARLDEAAWQALGARAGEHGLSAAGVALAAFAETIGRWS
QAPAFCLNLTVLNRPPLHPQLAQVLGDFLTALSLLAVDSRHGDSFVERARRIGEOMFDDLDHPTFS
GVDLLRELARRRRRGADLMPVVFSTSGIGSVQRLLDGDEAPRAPRYMISQTPQVWLDCQVTDQFGG

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LEIGWDVRLGLFPEGQAEAMFDDFVGLLRRLAQSPRAWTDGDATEPVEAPPQALPGSARSTIAGF
AERALLTPDATAIHDAAGSSYRQVAQHASALRRVLEAHGAGRGRRVAVMLPKSAAQLVAVIGIL
QAGAAYVPVDIRQPPPLRRQAILASAEVVALVCLESDVPDVGACVAIDRLAADSAPPPPAEVA
ADDLAYVIYTSGSTGTPKGVMLSHAAVSN'TLLDINQRYGVDANDRVLGLAELSFDLSYDFFGAT
AAGAQVVLDPARGSDPSHWAELLERHAITLWNSVPAQGQMLIDYLESEPQRHLPGRCVLWSGD
WIPVSLPTRWRRWPDALFSLGGATEAAIWSIEQPIRPQHTELASIPYGRALRGQSVEVLDARG
RRCPPGVRGEIHIGGVGLALGYAGDPQRTAERFVRHPDGRRLYRTGDLGRYLADGSIEFLGREDD
QVKIRGHRIELAELDAALCAHPQVNLAATVVLGETHERSLASFVTLHAPVEAGEDPRTALDAVRQ
RAAQALRRDWGSSEEGIAAAVAALDRACLASLAAWLAGSGLFASATPLDLATLCQRLGIAEARQRL
LRHWLRQLEEGGYLRAEGEGWLGAERPAQSPEDAWTAFAGCAPAALWPAELVAYLRDSAQSLGE
QLAGRISPAALMFPGSARIAEAMYSQGLHAQALHEAMAEIAAIVERQPQRRWRLLLELGAGTAA
ASRTVIARLAPLVQRGAEDVYLFDTVSSYFLAAARERFADQPWVRFRGFRDMNGDLLDQGVAPHSV
DILLSSGALNNALDTPALLAGLRELLSADAWLVIQELTREHNEISVSQSLMMENPRDLRDERRQL
FVHTGQWLEWLAAQGGDLACGVVPPGSALDLLGYDVLRLARCKTDRARLEPAELLAFAVEARVPRYM
LPAQLRVLERLPVTGNGKIDRKALTGFAQRPQADLRHGVQAQAPADELENALLALWREVLNPNLSLG
VEQDFFGAGGDSLLIAQLIARLRERLESARRHPFDRLLRWALSQPTPRGLAERLRSAPEEGRGPA
LAAARGVAPAPAGMSRAPLAEGAVALDPLVRLVPGEGVPRVLVHEGLGTLLPYRPLLRALGEGRP
LLGLAVHDSDAYLAIPAHLNACLGRRYAEALHRAGLREVDLLGYCSGGLVALETAKSLVQRGVR
VRQLDIVSSYRIPIRVDDERLLLFSAATLGLDTAALGFPAPELRLGQAVQAALAQTPERLVAEAL
AGLPGLADLVALRGRVLQAASGSADAVSVERDTLYRLFCHSVRASQAEAPEPYVGALRLFVPDAG
NPLVPRYAEALETQWRAAALGACGIHEVPGGHFDCLGEALAQSLSKPMPEEASR

```

MUT17

A *Pseudomonas* bacterial mutant (MUT17) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding putative ATP-binding component of the ABC transporter, *pchH* (PA4223). This gene encodes the VIR17 nucleic acid (SEQ ID NO:33) shown in Table 19A.

Table 19A. VIR17 Nucleotide Sequence (SEQ ID NO:33)

```

GTGACCCCGGTGCTGTGGCGCCTGCTGCGCACCTATCGCTGGCGGCTGGCGGCGGCCATGGGGTTGC
AGGCCCTGGCCGGGCTCTGCTCGCTGTTCGCCCTGGATGCTTCTCGCCTGGCTCGCCGAGCCGCTGGC
GCGCGGCCAGGCGCAGCCGGCCCTGCTGGCCCTGGTGCTGCTGGCGGTGCTGGCCTGGCTGGGCTGC
CAGGCGCTGGCCGCGCACCTGGCCACCGGGTCGACGCGGACCTCTGCAACGACCTGCGCCTGCGCC
TGCTGGCGCACCTGCAACGGCTGCCGCTGGACTGGTTCCGTCGCCAGGGCCCGGACGGCGTGGCGCG
CCTCGTGGAGCAGGACGTGCGGGCCCTGCACCAACTGATCGCGCACGCTCCCAACGATCTCAGCAAC
CTGTTGGTGGTGCCGCTCGTCGCGTTGCTCTGGCTGGCCTGGCTGCACCCCTGGCTGCTGCTGTTCT
GCCTGCTGCCGCTGGTGCTGGCCGCCGCCGGCTTCCTGCTGCTGCGCTCGGCGCGCTACCGCGACCT
GGTGCTGCGGCGCAACGCCGCGCTGGAAAGGCTCTCGGCGGACTATGGCGAATTCGCCACAACTG
CTGCTGGCCCGACAGTACCCCGGCGCCGGCATAACAAGGGCGCCGAGGCGTGGCGGCGGCCCTTCG
GCGAAGCGTTTCGGCGCCTGGGTGAAGCGGGTCGGCCACCTCGCCGCGCTGGTCTACGTGCAGTTGTC
GACGCCCTGGCTGCTGGCCTGGGTCTGCTCGGCGCGCTGGCCCTGGATGCCCTCGGCGTGCCGCTG
GCGCTCGGCCAGGCTGTGCTTCTGCTCCTGCTGCGGGCCCTGGCTGCCCGGCTGCAGGCGCTCG
GCCACGGCGCGCGCTGCTGGGCGCGCGCGCGCGCGCGCGAGCGCTGCAGCAGGTGTTTCGACCA
GGCGCGGCTGGCCGAGGGCCGCTCGACCCGCGAGCGGTCGATGGCGCGGTTGGCGCTGCACGCGCTG
GGCCATGCCTATGAAGCGCTGGAGGTCTGGCCGATATCGATCTGGAGCTGGAGGATGGCAGCCTGG
TGGCCCTGGTGGTCCCTCGGGCTCCGGCAAGAGCACCTGCTGCACCTGCTGGCGCGCTACATGGA
CGCGCAGCGCGCGCAACTGGAGGTGGCGGCCCTGGCACTGAAGGACATGCCTGATGCCGTGCGCCAT
CGGCATATCGCGCTGGTGGCCAGCAGGCGCGCGCGCTGGAGATATCCCTGGCCGACAACATTGCCCT
TGTTCCGCCCCGATGCCGATCTCCAGGAGATTCCGCCAGGCGCGCGCGCTGACGCGCTGCCCTGACGAGCG
CATCATGGCCCTGCCGCGTGGCTACGACAGCGTGCCGGGACGCGACCTGCAACTGTCCGGCGGCGAA
CTGCAACGACTGGCCCTGGCCCGTGGCTGCTATCGCCGCGGAGCCTGTTGCTGCTCGACGAGCCAA

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CCTCGGCGCTGGATCCGACACCGCCCGGCGAGGTCTGCGCAACCTGCGCGAACGCGGCGGTGGCCG
 GACCCGGGTGATCGTCGCCCATCGTCTGGCCGAAGTCAGCGATGCCGACCTGATCTGGTCTGGTC
 GCTGGCCGCTGGTCTGAACGCGGCGAGCACGCGGCGCTGTTGGCGGCGGACGCGCCTATGCGCGCT
 TGTGGCGTGAACAGAACGGCGCGGAGGTGGCGGCATGA

The VIR17 protein (SEQ ID NO:34) encoded by SEQ ID NO:33 is presented using the one-letter amino acid code in Table 19B.

Table 19B. Encoded VIR10 protein sequence (SEQ ID NO:34)

MTPVLWRLLRTRYRWRLAAAMGLQALAGLC SLLPWMLLAWLAEPLARGQAQFALLALVLLAVLAWL
 GCQALAAHLAHRVDADLCNDLRLRLLAHLQRLPLDWFGRQGPDGVARLVEQDVRALHQLIAHAPN
 DLSNLLVPLVALLWLAWLHPWLLLFCLLPLVLAAAGFLLRLRSARYRDLVLRRNAALERLSADYG
 EFAHNLLLARQYPGAGIQQGAEEASAAAFGEAFGAWVKRVGHLLAALVYVQLSTPWLLAWVLLGALA
 LDALGVPLALGQACAFLLLRALAAPVQALGHGGDALLGARAAAERLQQVFDQAPLAEGRSTREP
 VDGAVALHGLGHAYEGVEVLADIDLEEDGSLVALVGPSGSGKSTLLHLLARYMDAQRGELEVGG
 LALKDMPDAVRHRHIALVGQQAALAEISLADNIALFRPDADLQEIQAARDACLDERIMALPRGY
 DSVPGRDLQLSGGELQRLALARALLSPASLLLLDEPTSLDPQTARQVLRNLRERGGGRTRVIVA
 HRLAEVSDADLILVLVAGRLVERGEHAALLAADGAYARLWREQNGAEVAA

The role of VIR17 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT18

A *Pseudomonas* bacterial mutant (MUT18) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding the putative ATP-binding component of ABC transporter, *pchI* (PA4222). This gene encodes the VIR18 nucleic acid (SEQ ID NO:35) shown in Table 20A.

Table 20A. VIR18 Nucleotide Sequence (SEQ ID NO:35)

ATGACCCTGTTTGAACGAATGCGTGCGCTGCCCGAAGACTGCCCGTGCCGCGTTGCGCCGGGCGAGCG
 CCTGGGCGGTCTTGGCGGCGCTGCTGGACGCCGCTTGGCGCGTATTGCTGGTGCCGTTGGTCGAGGC
 CTGGTTTCGCCGAAGGCGCGTTGCCCTGGCGCTGGGTGCGCGCTTGCTCGGCTTGAGCCTGGCGCAG
 GCGCTGTTGAGTACCTGGCCCTGCGTTCGCGGTTTCGCCCGCGCGGCTCGCTGGCGGCTGGACTGG
 TGGCAGCCTGGTGGCGCGCTTGCCGCGCCTGGCGCGCGCGGCTGCGCGCGGCTGCGCGCGGCGCGA
 AGGCCTGCTGCGCGGCGCGGCGGTGATGACAGGCGATGGCGATTCCGGCGCACCTGCTGGGGCGCTGATC
 GCCGCGTTGGTGACGCCGCTCGGGGTGATCCTCGGGCTGTTCTGATCGACCCGTCATCGCCCTCG
 GCCTGCTCCTTGCTGGTGCCTTCCTCGCCGCGCTGTTGCGCTGGAGCGGGCGGCGCAATCTGGCGGC
 GGAGGATGCCCGGCTGGCCGCCGAGCGCGACGCCGACGGCAGTTGCAGGCGTTGCCCGAACGCCAG
 CCACTGCTGCGCGCGGCGCAGCGCGAAAGCGTCGCCCGCCAGGGGCTGGAAGAGGCCTTGCAGCAGTC
 TCCACGCGAGCACCTGGATCTGTTGCGGCGCAGCCTGCCAGCGGCCTCGGCTTCGCCCTGGCGGT
 GCAGGCGGCGTTGCGCTTCGCCCTGCTCGGCGCGCCTGGGCGGTGGAGCGGCAATGGCTGGACGGC
 GCTCGGCTGGTGGCGGTGCTGGTGCCTTCATCGAGCGGCTGGCCAGCTCACCACATC

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TCGACCAGGCGTTGCGCGGCGCCTGGCAGGCGCTGGATACCTGCTGCGGGTTTTCGCCCTGGCTCC
GCTGCGCAGCCCCGAGCCGGGCGAGCGGCGCACGACGCCAGCCTGGCGGGCGAGGCCGTGGAATTG
CGCCTGGAAGATGGCCGCGCCTTGCTCGAGGACATTTCCCTGAGGCTGGAGCCGGGTTGCTGAACG
TCCTCGTCGGACCTCCGGGGCCGGAAGAGCAGCCTGCTGGCGCTGCTCGGGCGGCTCTACGACGT
CGATGCCGGGCGTGTCTGCTGGGTGGCGTGGATATCCGCCGGTTGAGCGAAACGACCTCGCCGCC
AGTCGTAACCTGGTGTTCAGGACAACGGCCTGTTCCGCGGACGCTTGCCTGGAACCTGCGCATGG
CGCGAGCGGACGCCGATCTCGAAGCGCTGCGCGAGGCGGCGCGGGCGGTTGGCCTGCTGGAAGAGAT
CGAGGCCTGGCCGCGAGGGCTGGGACAGCGACGTCCGTCCCGGCGGCGCGCTGCTGTCCGGCGGCCAG
CGGCAACGCCCTGTGCCTGGCTCGCGGGCTGCTCTCGACGGCGCCGTTGCTGCTGCTCGACGAGCCCA
CCGCCAGCCTCGACGCCGCCAGCGAGGCGCAGGTGCTGCGCAGCCTGCTCGGGTTGCGCGGCCGGCG
CACCTGCTGGTAGTGACCCACCGCCCGGCGCTGGCGCGTCAGGCCGACCAGGTACTGCTGCTGGAG
GAGGGGCGCCTGCGCCTCAGCGGACTTCACGCCGATCTGCTCGTCCGGGACGACTGGTATGCCGGTT
TCGTCGGCTGGCGGGCGAGGAAAGTTCCGCGACGGTCTGTTGATCGATAG
```

The VIR18 protein (SEQ ID NO:36) encoded by SEQ ID NO:37 is presented using the one-letter amino acid code in Table 20B.

Table 20B. Encoded VIR18 protein sequence (SEQ ID NO:36)

```
MTLFERMRALPEDCRAALRRASAWAVLAALLDAACGVLLVPLVEAWFAEGALPWRWVAALLGLSL
AQALLQYLALRRGFAAGGSLAAGLVRSIVARLPRLAPPALRRVAPAEGLLRGPVMQAMGIPAHL
GPLIAALVTPPLGVLGLFLIDPSIALGLLLAGAFLLRWSGRRNLAAEDARLAAERDAARQLQ
AFAERQPLLRQAQRESVARQGLEEALRSLHRSTLDLLRRSLPSGLGFALAVQAAFAFALLGGAWA
VERQWLDGARLVAVLVLLVRFIEPLAQLTHLDQALRGAWQALDILLRVFALAPLRSPEPGERPHD
ASLAAEAVELRLEDGRALLEDISLRLEPGSLNVLVGSPGAGKSSLLALLGRLYDVDAGRVLLGGV
DIRRLSETTLAASRNLFQDNGLFRGSVAWNLRMARADADLEALREAAAVGLLEEIEAWPQGW
SDVPGGALLSGGQRQRLCLARGLLSTAPLLLLDEPTASLDAASEAQVLRSLGLRGRRTLLVVT
HRPALARQADQVLLLEEGRLRLSGLHADLLVRDDWYAGFVGLAGEESSATVVDR
```

The role of VIR18 in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

MUT19

A *Pseudomonas* bacterial mutant (MUT19) was made by transposon insertion in a *P. aeruginosa* wild-type strain PT894. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as a gene cluster encoding the *P. aeruginosa* serotype 09 putative O-antigen biosynthesis pathway (VIR19). The insertion site nucleic acid sequence identifying the VIR19 gene in MUT19 is shown in Table 21.

Table 21. MUT19 Transposon Insertion Site (SEQ ID NO:37)

```
CTCTTTCAGCCGCACGCGGCGCACCTCGTGTGTGATCAGTGAGTGGTTTGCAACTGCGGGTCAAG
GATCTGGATTTCCCTCACANGTNCGATCATCGTGCGGGAGGGCAAGGGCTCCAAGGATCGGGCCT
TGATGTTACCCGAGAGCTTGGCACCCAGCCTGCGCGAGCAGGGNNAATTGATCCGGTGGATGACC
```



```

TTTTGAATGACCTTTAATAGATTATATTACTAATTAATTGGGGACCCTANAGGTCCCCTTTTTTA
TTTTAAAAATTTTTTCACAAAACGGTTTATTTNCATAAAGCTTGCTCAATCAATCACCNTATCCN
CGGGAATTCGGCCTAGGCGGCCAGATCTGATCAAGAGACAGACCTCCAGCTTTGCATCCGGAGCG
ACCACACGAGCGAGGTCAGTCACTTTTCATCGAAGGAATTTTCTTGACATAGATCTCACCACCTTC
CATGTCTCTAAAGGCATGCCACACTAACTCGACGCCCTCCTCCAAAGAAATCATGAACCGGGTCA
TCCGCTCATCAGTGATAGGCAAGACGCCCTTGTCTTG

```

The role of this cluster in virulence was confirmed using phage to retransduce this mutation into the wild-type PT894 strain where attenuated virulence was again observed in the *Dictyostelium* growth assay compared to an isogenic bacterial strain.

B. Attenuated *Klebsiella* Mutants

MUT20

A *Klebsiella* bacterial mutant (MUT20) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding a hypothetical transcriptional regulator in met G-dld intergenic region (VIR20). The insertion site nucleic acid sequence identifying the VIR20 gene in MUT20 is shown in Table 22.

Table 22. MUT20 Transposon Insertion Site (SEQ ID NO:38)

```

ACGCAGGATATCTTCTTCATCAAATTGTCGATGCCCGCCTTCGCTACGCTGCGGTTTCAGTAGACCG
TAACGACGCTGCCAGGCGCGCAGTGTGACCGGATTGATTCCGCAACGTTTCGGCGACTTCACCGATAC
TGTAACACGCCATAGCAGCCTCACATCAACCTGATACCTTAATACCTAACTAACGAATTCAGGCAT
CCTGTACAACCTCTATTTTCTTGTACAGATAAAGATATCAGGTTGCGGCTCACAGCGCCCGGGAAAAA
AGATGAAAAAATGTTTAGCTGATTTCGCGGTGTTTCATTTTTTCTCCGGCCATGCGACGCGCGGTAG
GCCCCCAGGCGCGCGCTGGCGAACAAATTGCCCTGAAACTGTGAAATACCGGCTGATTCCAGCCAC
ATCCACTCTTCAGCAGCTCAACGCCGACGGCTGAGACCGCAATCTCCAGAGAAGTACAGCATTTGA
TAATCGCCTG

```

MUT21

A *Klebsiella* bacterial mutant (MUT21) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding γ -cystathionase (VIR21). The insertion site nucleic acid sequence identifying the VIR21 gene in MUT21 is shown in Table 23.

Table 23. MUT21 Transposon Insertion Site (SEQ ID NO:39)

GACCATGTGCTGATGACCAATACCGCCTATGAGCCAAGCCAGGACTTTTGTACCAAAATTCTCGCCA
 AACTCGGCGTCACCACCAGCTGGTTCGATCCCTTAATCGGCGCCGATATCGCCCGTCTGGTTCGCCC
 TGAGACCCGCGTGGTGTTCCTCGAATCGCCCGGCTCGATCACCATGGAAGTGCACGATGTGCCGGCG
 ATAGTCGCCCGCGTGCCTCAGGTCGCCCCGGAAGCGATTATCATGATCGATAACACCTGGGCGGGCGG
 GGATCCTGTTTAAAGCCCTGGATTTTGGCATTGATATTTCCATTCAGGCAGGCACCAAATACCTGAT
 CGGCCATTCCGACGCCATGGTGGGCACCGCGGTGGCGAACGCGCGCTGCTGGCCGCAGCTGCGTGAA
 AATGCCTACCTGATGGGGCAAATGCTGGACGCCGATACTGCCCTATATGACCAGCCGCGGCCTGCGAA
 CCCTGGGCGTGCCTGCGTCAGCATCATGAAAGCAGCCTGCGCATC

MUT22

A *Klebsiella* bacterial mutant (MUT22) was made by transposon insertion in a
 5 *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated
 microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide
 sequence immediately following the transposon insertion was cloned and identified as
 ribosome binding factor A (VIR22). The insertion site nucleic acid sequence identifying the
 VIR22 gene in MUT22 is shown in Table 24.

Table 24. MUT22 Transposon Insertion Site (SEQ ID NO:40)

CTTTTGGCCCTTTTTTGTCTTTATTCTGGAGAAGTATTATGGCGAAAGAATTTGGTCGCCCGCAG
 CGTGTGGCCAGGAGATGCAAAAAGAGATTGCCATCATCCTGCAGCGTGAAATTAAAGATCCGCGTC
 TGGGCATGATGACCACCGTTTCCGGTGTGGAAATGTCCCGTGACCTGGCCTATGCCAAGGTGTATGT
 CACCTTCCTTAACGACAAAGATGAAGCCGCGGTGAAAGCGGGCATCAAAGCGCTGCAGGAAGCTTCT
 GGCTTTATCCGCTCTCTGCTGGGGAAAGCGATGCGTCTGCGCATCGTACCGGAAGTACTTTCTTCT
 ACGACAACCTCACTGGTGAAGGGATGCGTATGTCCAACCTGG

MUT23

A *Klebsiella* bacterial mutant (MUT23) was made by transposon insertion in a
 15 *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated
 microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide
 sequence immediately following the transposon insertion was cloned and identified as the
 gene encoding aspartokinase/homoserine dehydrogenase (VIR23). The insertion site nucleic
 acid sequence identifying the VIR23 gene in MUT23 is shown in Table 25.

Table 25. MUT23 Transposon Insertion Site (SEQ ID NO:41)

GCCCAGCCCGCTTTCCCGCTTGCCAGTTAAAAGCCTTCGTGGAGCAGGAATTTGCTCAGATTAAGC
 ATGTTCTGCACGGCATCAGCCTGCTGGGTGAGTCCCGGACAGCGTCAATGCCGCGCTGATCTGCCG
 CGCGAAAAGCTCTCCATCGCCATCATGGCGGGTCTGCTGGAAGCCCGTGGACACAAAGTCAGTGTC
 ATTAACCCGGTCAAAAAGTCTCGCCGTGGGTCACTATCTGGAATCCACCGTCGATATCGCCGAAT

```
CCACCCGCCGCGATTGCCGCCAGCCAGATCCCGGCAGACCATATGATCCTGATGGCCGGGTTTACCGC
CGGCAATGAGAAAGGCGAGCTGGTGGTGCTGGGGCGTAACGGCTCCGACTACTCGGCTGCGGTACTG
GCCGCTGCGCTGCGCGCTGACTGCTGCGAAATCTGGACCGATGTCGACGGAGTGACACCTGCGGATC
CGCGTCAGGTGCCGGATGCGCGCTGCTGAAATCGATGTCTTATCAGGAGGCGATGGAGCTCTCCTA
CTTTGGCGCGAAAGTGCTGCACCCGCGCACCATTGCCCTATCGCCAGTTCCAAATCCCATGCCTG
ATTAATAATACCGGCAACCC
```

MUT24

A *Klebsiella* bacterial mutant (MUT24) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding cystathione • -synthetase (VIR24). The insertion site nucleic acid sequence identifying the VIR24 gene in MUT24 is shown in Table 26.

Table 26. MUT24 Transposon Insertion Site (SEQ ID NO:42)

```
GGCGCAGCGTCTGCTCGTCACCGTCAAGCTCGAAGCTTAACATTGCGCCAAAACCTTTTGTGACG
CGCCGCAATTTTCATGCCCTGGTTTTCCGGCAGCGATGGATGATACAGCTTTTTCACCAGCGGTGG
GTTTTTCAGATACTCAACGATCGCCAGGGCATTTCGCTGCGCCACTTCCATCCGTGGAGACAGCGTCC
GCAGCCCGCGCAACAGCAGATAGCTGTGCAAGGCGCTGCCGGTGACGCCAATATTATTGCCCCACCA
TGCCAGTTCCGTGACAGTTGCCGGATCTTTGGCAATCACCACCCCGGCCACCACATCGGAGTGACCA
TTGAGGTATTTGGTACAGGA
```

MUT25

A *Klebsiella* bacterial mutant (MUT25) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding phosphoribosylformylglycinamide synthase (VIR25). The insertion site nucleic acid sequence identifying the VIR25 gene in MUT25 is shown in Table 27.

Table 27. MUT25 Transposon Insertion Site (SEQ ID NO:43)

```
GTTGCGTCCCAGGCGGGTAAACGCATCCTGCAGGTAGTCAATTTTCGTCGTCGGCCAGCGCCAGACCC
AGACGGAGGTTGGCGTCAATCAGCGCCTGACGCCCTTCGCCCAGCAGGTCGACGCTGGTGACCGGCG
TCGGCTGATGGTGAGCGAACAGCTTCTCGCCCGCTTCAGCTCGTCGAAGACGCTCTCCATCATGCG
GTCATGCAGCTCCGCCGCCACCGCGGCCCACTGCGCTTCGGTCAGGGTTGAGGCTTCAACGTAATAC
GCCACGCCGCGCTCAAGACGCACAACCTGCGCCAGACCGCAGTTGTGAGCGATATCGGTAGCTTTAG
AAGACCAGGGAGAGATGGTGCCAGGGCGAGGGGTCACGAGCAGTAATTTACCGGTCGGGGTATGGCT
GCTTAAGCTCGGGCCATACTGAAGCAGTCGCGCCAGGCGCTCGCGATCGTCAGCGCTCAGCGGGGCG
TTCAGATCGGCAAAATGAATATATTTCGGCAT
```

MUT26

A *Klebsiella* bacterial mutant (MUT26) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding homoserine transsuccinylase (VIR26). The insertion site nucleic acid sequence identifying the VIR26 gene in MUT26 is shown in Table 28.

Table 28. MUT26 Transposon Insertion Site (SEQ ID NO:44)

```
GTATTGGCATCGTACTCCTGGGCTGGCCGGTGACAAAGGCGATGCGCTTATCTTTGCTGGCGAACAA
ATACGCATCGCCCTCTTCCGTCTCCGCGAGGATCTCGAGATCGGTATAGTCGCGAATAAGTCCGGCC
GGAAAATCAGCATAGCGTGAGTGCGGGGCCAGGAAAGAGTCGTCGAAACCGCGGGTCAGTAAGGCGT
GCGGATGAAGAATATGGTGTTTCATAGACGCCGGAATCTTTTCGGCGCGGGTCTGCTTGGGAATGCC
GTACAGAATGTTGAGCGCGGCCTGAACCGCCCAACAGACGAACAGCGTCGAAGTGACGTGATCCTTG
GCCCCACTCCAGCACCTGTTTGATCTGCGGCCAGTAAGCAACATCGTTAAACTCAACCAGGCCTAAAG
GAGCGCCGGTAACAATCAGGCCGTCAAAGTCTGATC
```

MUT27

A *Klebsiella* bacterial mutant (MUT27) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding 3'-phosphoadenosine 5'-phosphosulfate reductase (VIR27). The insertion site nucleic acid sequence identifying the VIR27 gene in MUT27 is shown in Table 29.

Table 29. MUT27 Transposon Insertion Site (SEQ ID NO:45)

```
GAGGTTTCATATGTCCGTACTCGATCTAAACGCGCTTAATGCATTGCCGAAAGTGGAACGCATTCTGG
CACTCGCGGAAACCAACGCCCAACTGGAAAAGCTTGACGCCGAAGGGCGTGCGCGTGGGCGCTGGA
AAATCTGCCGGGAAACTATGTGCTGTCGTCGAGCTTTGGCATTCAGGCGGCGGTAAGTTGCATCTG
GTGAATCAGATCCGCCCCGACATTCCGGTGATCCTCACCGATACCGGCTACCTGTTCCCGGAAACCT
ATCAGTTTATTGACGAGCTGACGGACAAG
```

MUT28

A *Klebsiella* bacterial mutant (MUT28) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the

gene encoding Sfi protein (VIR28). The insertion site nucleic acid sequence identifying the VIR28 gene in MUT28 is shown in Table 30.

Table 30. MUT28 Transposon Insertion Site (SEQ ID NO:46)

```
TGTTAAAGCGTGC GTTCTACAGCCTGTTAGTCCTGCTCGGCCTGCTGCTGTTGACCGTGC TGGGCCT
TGACCGCTGGATGAGCTGGAAAACCGCGCCCTATATCTATGATGAACGTCAGGACCTGCCCTACCGT
CAGGTCGGTGTGGTGTGCTGGGCACCGCCAAATATTACCGCACCGGCGTCATCAATCAGTATTACCGTT
ACCGCATCCAGGGTGC GTGTAACGCCTACAACAGCGGCAAGGTCAACTATCTCCTGCTGAGCGGCGA
TAATGCTCTGCAAAGCTACAATGAACCGATGACCATGCGTCGGGACCTGATTAAAGCGGCGCTCGAT
CCCGCGGATATCGTACTGGACTATGCCGGTTTCCGTACCCCTCGACTCGATCGTCCGTACCCGGAAG
TGTTTCGACACCAACGACTTCATTATCATCACCAGCGCTTCCACTGCGAACGGGCGCTGTTTATCGC
CCTGCATATGGGGATCCAGGCCAGTGCTACGC
```

5 MUT29

A *Klebsiella* bacterial mutant (MUT29) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding transcriptional activator protein LysR (VIR29). The insertion site nucleic acid sequence identifying the VIR29 gene in MUT29 is shown in Table 31.

Table 31. MUT29 Transposon Insertion Site (SEQ ID NO:47)

```
CGCTGAACCTCCTCAAACAAACGCGAGGCCCTGCACCTGTGCGCTGCAGGCGACCAGCGTGGATCCGC
TCAAACAGCTGCAGGCCGAGCACCTTCTCAAAGCGCGCCAGCTCGCGGCTGACCGTGGGTTGCGAGG
TGTGCAGCATCCGCGCCGCTTCGGTCAGGTTGCCGGTGGTCATCACCGCGTGAAAGATTTTCGATATG
ACGCAAATTGACGGCTGGCATGCGGTCTCCGTGAGGCTCGGCTGGAACCATATCATTTTTGCATAGA
GTCGCGATAAAACGATATTTTTTATTCGTCTGTCACTGTGGCGTAATCAGAAAAAACAGCGACCAAC
ACACGCACTGCACCGGAGTTCTTATGCCCACTCGCTTTACGCCACCGATACTGACCTGACCGCGGA
CAACCTGCTGCGCCTGCCGGCGGAATTTGGCTGCCCGGTCTGGGTCTATGATGCGCAGATTATTGCG
CGCCAGATAGCCAGCTCAGCCAGTTTCGAC
```

MUT30

A *Klebsiella* bacterial mutant (MUT30) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding TrpD (VIR30). The insertion site nucleic acid sequence identifying the VIR30 gene in MUT30 is shown in Table 32.

Table 32. MUT30 Transposon Insertion Site (SEQ ID NO:48)

GGCTTCCACCCAAATCGCTTTGTCTGGCAACGATTTTGTCTAAAACGGCTTTGCATTCTTTACCTCT TGCCCGCTAAGTGCGGTCACTCTGTCATAGGCCGCGCCGCTGCTGCAGCACATCCAGTACCTGCTGA GCGTTAGCTTTTCTAGATCTTCATGCCCCGTGTAAACGCATCAATATGGCGACGTTGGCGGCGACGGCGG CTTCGTGAGCGGCTTCACCTTTACCTTG
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MUT31

A *Klebsiella* bacterial mutant (MUT31) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding N-acetylglucosamine-6-phosphate deacetylase (VIR31). The insertion site nucleic acid sequence identifying the VIR31 gene in MUT31 is shown in Table 33.

Table 33. MUT31 Transposon Insertion Site (SEQ ID NO:49)

TGGCTCAACGCTGCTCAGTGCGTGGTGCAGGTTGTCACTTTGGTGATCACATCGGCGTTGTCTGCACAGTG AAATCAGATCCAGCGCCGCGTCCGGTTTTACGCACGTAGTCCGGATTGTGGGTGCCTTTCTTAACGA TATTCAGCCACGGCCCTTCGAGATGCAGGCCAGCGCCTGGTTCGGATGTTTTGCAGATATTCGCG CATCACGCGCACGCTTGTCTCATCAGATCGTCGCTGGAGGTAATCAGCGTCGGCAGGAAGCTGGTG CAGCCTGAGCGTTGCTTGGCCTTCTGCATGATCTCCAGCGTTTCGACAGTGACCGCTCTGGGCTGT CGTTAAACTGCACGCCGCCGAGCCGTTGAGCTGGACGTCGATAAAACCGGGGGCGATTATTGCGCC GTTGACTGAGCGCTGCTCGATGTCAGACGGCAAATCTGCCAGCGGACAAAGACGTTTCGATAAAG
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MUT32

A *Klebsiella* bacterial mutant (MUT32) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding WaaQ (VIR32; Regué *et al.* J. Bacteriol. 183(12): 3564-73, 2001). The insertion site nucleic acid sequence identifying the VIR32 gene in MUT32 is shown in Table 34.

Table 34. MUT32 Transposon Insertion Site (SEQ ID NO:50)

TTAAGCACCATATCGTACCGCTGCTGGCGCAGCGTCTGAATGAGCTGCCATTGCATCTTCAGCTGAT ACCTTTTTCCCTGGCTTTTTCCAGCGGCGATCGAGACCATAAATATGGTGGATATCGGGGTTGGCTG CGAGCATATCCCGGGTCTCTTCATAACAACAGGACATCCACGCTGGCGGGGGTACTGCTGTTTCAG CGCGTGAATAAGCGGGGTGATCAGCAGCATGTGCCATGATGGCGCAGCTTAATGACCAGGATCCGC GCCGGGTTCAACGGGGCCGCGGGAGAGGGTTTTAGGCGTCATACTCTGTTCTTCATCCAGGATAAGGG TTCCGATTCTAGGGGATCAGACAGATTGAGAGAAGCGTTGTATTGCTCTACCATGACCCGATACGTA TGGCCTGAGGACGTTTTCTGTCACAATCCCGCAATTTCTCATCAGAT
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MUT33

A *Klebsiella* bacterial mutant (MUT33) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding 2-isopropylmalate synthase (VIR33). The insertion site nucleic acid sequence identifying the VIR33 gene in MUT33 is shown in Table 35.

Table 35. MUT33 Transposon Insertion Site (SEQ ID NO:51)

```
CACTCAGGCTTGCCTGTAACGCTTGTTCGCCATCACGTAAGGTCGTATCGAAAATAATGACTTGCTG
GCTCATGGTTTGGATCCTTAGTCTGTGTCCTGGCGCCTTGTGACGAGCATAAAAAACCCGCGCCA
AGGCGCGGGTTTATAGTCTTGCTGGAAGATGACTTAACGCTGAACGTCGCCAACAGCCTACCGAG
CAAATGGCATGCGTTTAGTAGTAGTAGGCTGGTGATACGAGCGGTGCGAATCATTGCGTCAAACCTCC
AGATGAAATCGTTATGCTTTTAGAGTTACTGGATAGCCGTTTTAAAGTCAACCCCTGGCATGGAAAA
AGCGTTTTGGGCTGACTAAATGAATTAGCAAAATGTGCTGATGTAAGCCCCATTTTGCCGAAGATCC
TATTTTGGACCGAAGGCGGTTTATCCCAATTGTTTCATTGAAAAA
```

MUT34

A *Klebsiella* bacterial mutant (MUT34) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding histidinol dehydrogenase (VIR34). The insertion site nucleic acid sequence identifying the VIR34 gene in MUT34 is shown in Table 36.

Table 36. MUT34 Transposon Insertion Site (SEQ ID NO:52)

```
CGCTGAACCGCTATCCGGAGCCGCAGCCGAAGTGCCGTGATTGAGAGCTACGCCCGCTACGCCGAGG
TCAAACCGGAGCAGGTGCTGGTCAGCCGCGGCCGACGAAGGCATCGAGCTGCTGATCCGCGCCTT
CTGTGAGCCCGGCGAAGACGCGGTGCTCTACTGCCCGCCGACCTACGGCATGTACAGCGTCAGCGCC
GAGACCATCGGCGTCGAGTGCCGCACCGTGCCGACGCTGGCCAGCTGGCAGCTCGACCTGCCGGGCA
TCGAAGCGCGGCTGGACGGCGTGAAGGTGGTGTGTTGCTGTCAGCCCGAACAACCCGACCGGGCAGAT
TATCGACCCGACGTCGATGCGCGACCTGCTGGAGATGACCCGCGGCAAGCCATCGTGGTGGCCGAC
GAAGCCTATATTGAATTCTGCCCGCAGGCACGCTCGCCGGCTGGCTCAGCGACTATCCGCACCTGG
TGGTGCTGCGCACGCTGTCCAAAGCCTTCGCCCTCGCCGGCCTGCGCTGCGGCTTCACCCTCGCCAA
CGCCGAGGTGATTAACGTGCTGCTGAAAGTGATCGCCCC
```

MUT35

A *Klebsiella* bacterial mutant (MUT35) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding UDP-galactopyranose mutase (VIR35; Clarke *et al.*, J. Bacteriol., 177: 5411-18, 1995). The insertion site nucleic acid sequence identifying the VIR35 gene in MUT35 is shown in Table 37.

Table 37. MUT35 Transposon Insertion Site (SEQ ID NO:53)

```
CGTATATTTTCATCGTACAGAAACCGTAAACACAGGCATTGGCTGATTTTCAGTGAGTGAATTTAAAT
AGACTTCTGCCGTTTTCAATGCTTCGGCGATGGTCACATCCATATCAAGGTAACGGTAGGTTCCAAG
ACGACCGACAAAAGTGATGTTGGTTTCATTCTCGGCCAATGACAAATATTTTTCAAGAAGAGCCATT
TCTCCCATCTGGCGAATAGGATAGTAAGGAATATCATTTCCTTCACAAGCACGGCTATACTCTTTAT
AACAAACAGAGCCGTCGTGTTGTTCCAGGGAGAAAAATATTTATGTTTCAGTGATGCGAGTATAGGG
CACATCCACAGAACAGTAGTTCATCACTGCGCATCCCTGG
```

MUT36

A *Klebsiella* bacterial mutant (MUT36) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding O-antigen export system permease protein rfba (VIR36; Bronner *et al.*, Mol. Microbiol., 14: 505-19, 1994). The insertion site nucleic acid sequence identifying the VIR36 gene in MUT36 is shown in Table 38.

Table 38. MUT36 Transposon Insertion Site (SEQ ID NO:54)

```
GTACGCCGATTTTATATGCGTCTGATATGATTCCGGAAAAATTTAGCTGGATAATTACCTACAATCC
GCTAGCGAGTATGATTCTTAGTTGGCGTGATTATTCATGAATGGGACTCTTAATTTGAGTATATT
TCTATACTCTATTTACGGGAATTATTTTGACGGTTGTCGGTTGTCTATTTTCAATAAATTTAAAT
ATCGATTTGCAGAGATCTAAAAGTGCCTATAAGAGCAGCATGCTAGGCTATTTATGGTCAGTAGCA
AATCCATTGCTTTTGGCATGATTTACTATTTTATATTTAAGCTGGTAATGAGAGTACAAATTCCAA
ATTATACAGTTTTCCTCATTACCGGCTTGTTCCGTGGCAATGGTTTGCCAGTTTCGGCCACTAAC
```

MUT37

A *Klebsiella* bacterial mutant (MUT37) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated

microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding uridylyltransferase (VIR37). The insertion site nucleic acid sequence identifying the VIR37 gene in MUT37 is shown in Table 39.

Table 39. MUT37 Transposon Insertion Site (SEQ ID NO:55)

```
CGAGCCACCCACTGTAGCGTATGGATATCGCGCAAGCCGCCGGGGCTGCTTTTCACGTCCGGCTCGA
GGTTATAGCTGGTGCCATGATAGCGCTGATGACGGACGTTCTGCTCTTCGACCTTGGCGGCGAAGAA
CTTTTCCGATGGCCAGAAGCCGTCGCTAAAAATATGTTTTTGCAGTTCAAGGAACAGCGCGACGTCG
CCGATCAGCAGGCGCGATTTCGATTAAAGTTGGTGGCAACGGTCAGATCCGAGAGACCTTCCAGCAGGC
ACTCTTCGAGGGTGGCTACGCTGTGGCCACCTCCAGCTTGACGTCCACAGCAGGGTGAGCAGTTC
GCCGACTTTTTTGCGCCCTGGTCGTCCGGCAGTTTTTTACGACTGAGGATCAGCAGATCGACGCTTGAG
AGCGGGTGCGAG
```

MUT38

A *Klebsiella* bacterial mutant (MUT38) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding pyridoxine phosphate biosynthetic protein PdxJ-PdxA (VIR38). The insertion site nucleic acid sequence identifying the VIR38 gene in MUT38 is shown in Table 40.

Table 40. MUT38 Transposon Insertion Site (SEQ ID NO:56)

```
CTTAACCCGACGCTGGCGAAGGCGGCCATATGGGAACAGAAGAGATAGACACCATCATTCGGGTGC
TGGAAGAGATGCGCGCAAAGGGGATGAACCTCAGCGGTCCGCTGCCGGCAGACACTCTCTTTCAGCC
GAAATATCTTGATCATGCCGATGCGGTACTCGCGATGTACCACGATCAGGGCCTGCCCGTGCTAAAA
TACCAGGGCTTTGGCCGCGGCGTGAACATTACGCTCGGTTTACCTTTTATTCGTACCTCCGTCGACC
ACGGCACC GCACTGGAATTAGCGGGCCAGGGAAAAGCGGACGTCGGCAGTTTTATCACGGCGCTTAA
TCTCGCCATCAAAATGATTGTTAATACCCAATGAATAATCGAGTCCATCAGGGCCATTTAGCCCGCA
AACGCTTTCGGGCAGAACTTCCTCAACGATCAGTTTGTGATCGACAGCATCGTCTCGGCGATTAAACC
GCAGAAAGGCCAGGCGATGGTTGAAATCGGC
```

MUT39

A *Klebsiella* bacterial mutant (MUT39) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding triose phosphate isomerase (VIR39). The insertion site nucleic acid sequence identifying the VIR39 gene in MUT39 is shown in Table 41.

Table 41. MUT39 Transposon Insertion Site (SEQ ID NO:57)

```
GGGTCTGACCCCGGTTCTGTGCATCGGTGAAACCGAAGCCGAAAACGAAGCGGGCAAAACGGAAGAA
GTTTGCGCACGTCAGATCGACGCCGTGCTGAAAACCCAGGGCGCTGCCGCTTTCGAAGGCGTGGTTA
TCGCTTACGAACCAAGTATGGGCTATCGGTACCGGCAAATCAGCGACCCCGGCTCAGGCGCAGGCGGT
GCACAAATTCATCCGTGACCACATTGCTAAAGCTGACGCCAAAATCGCTGAGCAAGTGATCATCCAG
TACGGCGGTTCCGTTAACGCTGGCAACGCCGAGAGCTGTTACCCAGCCGGACATCGACGGCGCGC
TGGTTGGCGGCGCCTCCCTGAAAGCTGACGCTTTCGCGGTGATCGTTAAAGCAGCAGAAGCAGCGAA
AAAAGCGTAATTCGCTTTTCCCGGTGGCGACACGCGACCGGGTTGACTGACAAAACGTGGGAGCCCG
GCCT
```

MUT40

A *Klebsiella* bacterial mutant (MUT40) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding aldehyde dehydrogenase (VIR40). The insertion site nucleic acid sequence identifying the VIR40 gene in MUT40 is shown in Table 42.

Table 42. MUT40 Transposon Insertion Site (SEQ ID NO:58)

```
GGTGGCGCACCTGGCGTCGTTTGTGTAGAAATATGAATATTAATACCAGGAAAATTCCTAATTTT
TGTGTACGCTCTGACGAGCGCACATAAAACAAGACGAATTTTGAACAATTGCTTTTAAATTTGTT
AATTGAATTGATCTGTTGTTGTTTAAAGGTATTTGAATTTCTTTTGTATAGATATGTAAATTAACAT
TGAAAAGCCATTTCAAAAATTAAATATATGGCGAACATAGCTATTAAGTTATAGTTAACATCTTCCC
GGGTTGCCTTTTGATACTTCGGGTAATATATTTATTTTCGCACATCAAAATAACTCTTTTTTCTTCTG
TTTGTTATTTCATGGCCATCTATTGGCGAAATAAGGCAGAGTAGAGGGGGATGTGCCTAATATCCTGC
GGAAGGAACGCAATGTACATTTACAGGGAGGAGCTGACGAGCCGTTTCGCGATAGCTTTAG
```

MUT41

A *Klebsiella* bacterial mutant (MUT41) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding galactosyl transferase (VIR41; Clarke *et al.*, J. Bacteriol., 177 : 5411-18, 1995). The insertion site nucleic acid sequence identifying the VIR41 gene in MUT41 is shown in Table 43.

Table 43. MUT41 Transposon Insertion Site (SEQ ID NO:59)

```
TTGGTGGTGTGCTCGCGAAGAAATTTAATCTGCCGGTCATCGTAAGTTTTGTTGGGCTTGGAAGAGT
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ATTTTCTTCTGACAGCATGCCTTTAAATTATTGCGGCAGTTTACTATTGCTGCATATAAATATATT
GCCAGTAATAAGCGCTGTATATTTATGTTTGAACATGACCGCGACAGAAAAAACTGGCTAAGTTGG
TTGGACTCGAAGAACAACAGACTATTGTTATTGATGGTGCAGGCATTAAATCCAGAGATATACAAATA
TTCCTTTGAACAGGATCACGATGTCCCTGTTGTATTGTTTGGCAGCCGTATGTTGTGGAGTAAAGGA
CTGGGCGACTTAATTGAAGCGAAGAAAATATTACGCAGTAAGAATATTCACTTTACTTTGAATGTTG
CTGGAATTCGGTCGAAAATGATAAAGATGCAATTTCCCTTCAGGGTCATTGAAAATTGGCATCAGC
AAGGATTAATTAAC TGGTTAGGTCGTTTCAATAATGTTTGCATCTTATTGAGCAAT

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MUT42

A *Klebsiella* bacterial mutant (MUT42) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding siroheme synthetase (VIR42; Kolko *et al.*, J. Bacteriol., 183 : 328-35, 2001). The insertion site nucleic acid sequence identifying the VIR42 gene in MUT42 is shown in Table 44.

Table 44. MUT42 Transposon Insertion Site (SEQ ID NO:60)

```

T T A C T T G C C C C T T T T T G C C G A A C T G A A A C A A A G G C C C G T G C T G G T G A T C G G C G G C G G C G A G A T T G C T
G A A C G T A A G A T C A A G T T C C T G C T G C G C G C C C A G G C G C A G G T G C A G G T G G T C G C T G A A A C G C T G T C A C
C G G C G C T G G C C G A T C T G G C T G C G C G C C A G G C A C T C A G C T G G C G G G C G A C G G C A T T C A G C G A C T C G C T
G G T G G A T G A T G T C T T T C T G G T G A T T G C G G C C A C C G A G G A T G A G G C G C T T A A C C A G C G G G T G T T T G C G
G C A G C T A A C G C G C G C T A C C G G T T G G T C A A C G T G G T G G A T A A C C A G G C G C T G T G C T C G T T T G T T T T C C
C T T C T A T C G T C G A C C G T T C G C C G C T G C T G G T G G C G A T C T C C T C A G C G G T A A A G C G C C G G T G T T G T C
G C G C A T T C T G C G T G A A A A A T C G A A G C G C T G C T G C C G A C G A A T C T C G G T C G G C T G G C G G A A T C A G C A
A G C T

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MUT43

A *Klebsiella* bacterial mutant (MUT43) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding 7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (VIR43). The insertion site nucleic acid sequence identifying the VIR43 gene in MUT43 is shown in Table 45.

Table 45. MUT43 Transposon Insertion Site (SEQ ID NO:61)

```

A G C A G G G C A A T G G T G G T C G G T T T C A T A A C A T T T C C T G A T G A T G A A A G T C A T A T T A A C C G G C A T T C T A
A C A G C A G C A T T C A G A G G G C A A T G A T T T T G G G C A A C C G A T T A C G A C G A T C G C C G C A A A T G C T A A A A
A G G G A G A G G G G A T T A C C A G C T G G C G G G C T T T T C C G C G C C G A G A T T A T C C A G C A C G G C G C G C A G C G C C
A G G C C G T C A G G A A G T G A A G G T C C G G G G C G A T C T C G A A C A G C G G C C A G A G C A T A A A G C C G C G G T T T T

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TCATATCGTAGTGCGGAACGGTCAGGCGCTCGCTGTTAATGACAGCATCGCCAAACAGCATGATATC GAGGTCCAGCGTGCGCGGCCCCAGCGTTTCGGCTTTGCGCACTCGCCCCCTGCTGCAGTTCGATGCGC TGAGTATGATCGAGCAGCGTCTCGGGGGGCAGGGCGGTTTCCAGCGCAA
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MUT44

A *Klebsiella* bacterial mutant (MUT44) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding glucose-6-phosphate isomerase (VIR44). The insertion site nucleic acid sequence identifying the VIR44 gene in MUT44 is shown in Table 46.

Table 46. MUT44 Transposon Insertion Site (SEQ ID NO:62)

GGCTTAACGCCAGCTATGTCAACGCTGCGGTTATGCGGATTTTTCATGCCTCTGCGGCTAACAGAAA AAAGCCTTATGATAGCTATACTAATGGGGCTTTTACTCCGTTTTGACCCGATTCTTGACCGGCGTC AGGGTCAAGTCACAAAAATCATCACAATTTTCGCTACCGGCGCTACAATCGACCGAAGTCACAATC TCAAATCAGAAGAGTATTGCTAATGAAAAACATCAACCCAACGCAGACCTCTGCCTGGCAGGCATTA CAGAAACACTTCGACGAAATGAAAGATGTCATATCAGCGAGCTTTTCGCCAAAGATAGCGACCGTT TTTCTAAATTTTCCGCGACGTTTCGACGATCTGATGCTGGTGGACTTCTCCAAAAACCGCATCACTGA AGAGACGCTGGCTAAACTGCAGGATCTGGCGAAAGAGACTGACCTGGCGGGCGCTATCAAGTCGATG TTCTCAGGTGAGAAGATCAACCGCACCGAAGACCGCGCGGTACTGCACGTCGCGCT

MUT45

A *Klebsiella* bacterial mutant (MUT45) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding DNA methylase (VIR45). The insertion site nucleic acid sequence identifying the VIR45 gene in MUT45 is shown in Table 47.

Table 47. MUT45 Transposon Insertion Site (SEQ ID NO:63)

TGCTTCATCCGCATCTCCTTGAAATTTATTTGGTCTTAGGCGGACGGTAGAGCGCTAATAGCTCGTC CACCTTTTTACGCGTACCACCGTTGCTGCTGATGCTGCGCCGCACCTTCACAATATGCGTTTCTGCC GCGTTTTTATACCATTCCTGCGTCAGCGGCGTGCGGTGGTTGGAAATCAGCACCGGGATGCGCTTTT TCATCAGCGATTCCGCCCTTTGCGCCAGCAGTACCTGTTGTTCCAGGTTGAAACTGTTGGTGTGGTA GGCGGTAAAGTTCGCCGTCGCCGTTAGCGGCGCATAGGCGGATCGCAATACACCACTGTGCGGCTA TCCGCACGTTGCATGCACCTCTCGTAAGATTGCGAGTAAACTCGGCGTTTTCGCGCTTCTCGGCGA AATGATAGAGCTCAGCTTCGGGGAAATAGGGCTTTTATAACGGCCAAACGGCACATTGAACTCGCC GCGCAG
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MUT46

A *Klebsiella* bacterial mutant (MUT46) was made by transposon insertion in a *Klebsiella* sp. wild-type strain. In the *Dictyostelium* growth assay, the mutated microorganism was less virulent compared to an isogenic bacterial strain. The nucleotide sequence immediately following the transposon insertion was cloned and identified as the gene encoding a putative inner membrane protein (VIR46). The insertion site nucleic acid sequence identifying the VIR46 gene in MUT46 is shown in Table 48.

Table 48. MUT46 Transposon Insertion Site (SEQ ID NO:64)

TGTC AATGCGCAATTTGGTTAAATATGTCGGTATTGGCCTGCTGGTGATGGGGCTTGCCGCCCTGCGA TAACAGCGATTCAAAAGCGCCAACCGTTGGCGCAGCAGCGGAGAGCAATGCCAGCGGCCAGGCAATC AGCCTGCTGGATGGCAAGCTGAGCTTCACCCCTGCC'FGCGGGCATGGCCGACCAGAGCGGCCAAACTGG GTACCCAGGCGAACAATATGCACGTCTACTCTGACGCTACCGGCCAGAAAGCGGTCATCGTCATCGT CGCGGACAGCACCAATGA

10 IV. SUITABLE TARGET PATHOGENS

Other *Pseudomonas* sp. and *Klebsiella* sp. and many other microbes, including gram-negative bacterial strains, are likely to include virulence genes encoding VIRX-related peptides or proteins having amino acid sequence identity or similarity to those identified herein. Suitable bacterial pathogens may include, but are not limited to, *Pneumococci* sp.,
15 *Klebsiella*, sp., *Pseudomonas*, e.g., *P. aeruginosa*, *Salmonella*, e.g., *Salmonella typhimurium*, *Legionella*, e.g., *Legionella pneumophila*, *Escherichia*, e.g., *Escherichia coli*, *Listeria*, e.g., *Listeria monocytogenes*, *Staphylococcus*, e.g., *Staphylococcus aureus*, *Streptococci* sp., *Vibrio*, e.g., *Vibrio cholerae*. Pathogenic mycobacteria of the present invention may include e.g., *Mycobacterium tuberculosis*. Pathogenic fungi of the present invention may include,
20 e.g., *Candida albicans*. Pathogenic unicellular eukaryotic organisms of the present invention may include, e.g., *Leishmania donovani*.

Having identified VIRX genes according to the invention, it is possible to use the gene sequence to search for related genes or peptides in other microorganisms. This may be carried out by searching in existing databases, e.g., EMBL or GenBank. The levels of
25 identity between gene sequences and levels of identity or similarity between, amino acid sequences can be calculated using known methods. In relation to the present invention, publicly available computer based methods for determining identity and similarity include the BLASTP, BLASTN and FASTA (Atschul *et al.*, J. Molec. Biol., 1990; 215:403-410), the

BLASTX program available from NCBI, and the Gap program from Genetics Computer Group, Madison WI.

Preferably, the peptides that may be useful in the various aspects of the invention have greater than a 40% similarity with the peptides identified herein. More preferably, the peptides have greater than 60% sequence similarity. Most preferably, the peptides have greater than 80% sequence similarity, *e.g.*, 95% similarity. With regard to the polynucleotide sequences identified herein, related polynucleotides that may be useful in the various aspects of the invention may have greater than 40% identity with the sequences identified herein. More preferably, the polynucleotide sequences have greater than 60% sequence identity. Most preferably, the polynucleotide sequences have greater than 80% sequence identity, *e.g.*, 95% identity.

In addition to related molecules from other microorganisms, the invention encompasses modifications made to the peptides and polynucleotides identified herein which do not significantly alter the biological function. It will be apparent to the artisan that the degeneracy of the genetic code can result in polynucleotides with minor base changes from those specified herein, but which nevertheless encode the same peptides. Complementary polynucleotides are also within the invention. Conservative replacements at the amino acid level are also envisaged, *i.e.*, different acidic or basic amino acids may be substituted without substantial loss of function.

It is recognized in the art that highly refined mechanisms that regulate transcription have evolved and are present in bacteria. Most bacterial genes are organized into operons, which are groups of genes coding for related proteins. Operons can either be repressed or induced thus regulating those genes. An operon consists of an operator, promoter, regulator, and structural genes. The regulator gene codes for a repressor protein that binds to the operator, obstructing the promoter (thus, transcription) of the structural genes. The regulator does not have to be adjacent to other genes in the operon. If the repressor protein is removed, transcription may occur.

Transposon mutagenesis usually inactivates the gene in which the transposon is inserted, as well as any gene downstream in the same operon. If the VIRX gene is a structural gene in an operon, inactivation of the VIRX gene disrupts the expression of other structural genes in the same operon and positioned downstream of the inactivated VIRX gene. For example, an insertion in *pchE* gene also inactivates *pchF*, *pchG*, *pchH*, and *pchI* genes

because they all reside within the pchEFGHI operon and are downstream of the inactivated pchE gene. Accordingly, the present invention includes attenuation of virulence due to alteration of a VIRX gene residing in an operon as well as alterations to nucleic acid yielding loss of expression of structural genes located in the same operon and located downstream of the VIRX gene. In one embodiment, the present invention is an alteration inactivating the first gene of an operon carrying a VIRX gene of the invention. The alteration of nucleic acids of VIRX genes and VIRX-containing operons may be insertional inactivation or gene deletion. It is preferred that the alteration of nucleic acids of VIRX genes and VIRX-containing operons be insertional inactivation.

The present invention also provides for a bacterial strain comprising an operon encoding a gene selected from the group consisting of VIR1, VIR2, VIR3, VIR4, VIR5, VIR6, VIR7, VIR8, VIR9, VIR10, VIR11, VIR12, VIR13, VIR14, VIR15, VIR16, VIR17, VIR18, VIR19, VIR20, VIR21, VIR22, VIR23, VIR24, VIR25, VIR26, VIR27, VIR28, VIR29, VIR30, VIR31, VIR32, VIR33, VIR34, VIR35, VIR36, VIR37, VIR38, VIR39, VIR40, VIR41, VIR42, VIR44, VIR45, and VIR46, wherein the bacterial strain includes a mutation that reduces expression of the VIRX gene relative to an isogenic bacterial strain lacking the mutation. In one embodiment, the mutation reduces inhibition of *Dictyostelium* amoeba growth when compared to the growth of *Dictyostelium* amoeba in the presence of an isogenic bacterial strain lacking the mutation. In another embodiment, the attenuated bacterial strain has more than one mutation of an operon containing a VIRX gene when compared to an isogenic bacterial strain.

V. VIRX NUCLEIC ACIDS AND POLYPEPTIDES CAN BE USED TO IDENTIFY ANTIMICROBIAL DRUGS

A. Screening

In a separate embodiment, the VIRX genes, or their polynucleotide or polypeptide products disclosed herein is used in screening assays for the identification of potential antimicrobial drugs. Routine screening assays are known to those skilled in the art, and can be adapted using the VIRX products of the invention in the appropriate way. For example, the products of the invention can be used as the target for a potential drug, with the ability of the drug to inactivate or bind to the target indicating its potential antimicrobial activity. In the

methods of the present invention, one or more test compounds may be present or produced in the assay mixture. Preferably one compound is present, or produced, in the assay mixture.

B. Character of Antimicrobial Candidate Compositions

5 VIRX nucleic acids and polypeptides may be used to identify drugs or therapeutics in a candidate composition useful in the prevention or treatment of pathogen-associated disease or infection. A candidate composition can include one or more molecules for analysis in a screening assay and can be a synthetic or semi-synthetic molecules. Such molecules include inorganic as well as organic chemical molecules. The molecules may be less than about 500
10 Daltons or more than 500 Daltons. The molecules may be naturally occurring. Naturally occurring molecules may include, *e.g.*, saccharides, lipids, peptides, proteins, nucleic acids, or combinations thereof, *e.g.*, aminoglycosides, glycolipids, lipopolysaccharides, or macrolides. Proteins may be immunoglobulins, *e.g.*, polyclonal or monoclonal antibodies. Nucleic acids may be DNA or RNA, *e.g.*, small interfering RNA (siRNA). The precise source of the
15 molecule is not critical to the method of the present invention. The molecule might be derived from *e.g.*, synthetic compounds libraries that are commercially available, *e.g.*, Sigma-Aldrich (Milwaukee, WI), or libraries of natural occurring molecules in the form of bacterial, fungal, plant, and animal extracts such as those available from Xenova (Slough, UK). The synthetic (or semi-synthetic) or natural occurring molecules might be modified using standard
20 chemical, physical, or biochemical methods known in the art.

VI. VIRX NUCLEIC ACIDS AND POLYPEPTIDES CAN BE USED TO DETECT THE DEGREE OF VIRULENCE OF PATHOGENS

A diagnostic test can assist physicians in determining the type of disease and
25 appropriate associated therapy. As such, a separate embodiment of this invention provides for the use of VIRX genes or their polynucleotides or nucleic acid products as virulence markers for detecting the presence of a pathogen, a pathogen-associated disease, or the virulence of a pathogen. There are many diagnostic assay approaches known to the artisan. Generally, the diagnostic method used would comprise the steps of (a) obtaining a sample
30 from a potentially diseased subject or a diseased subject; (b) measuring the level of at least one polypeptide or polynucleotide virulence marker in the sample; and (c) comparing the amount of the virulence marker in the sample of step (a) to the amount of the virulence

marker present in a control sample from a second subject known not to have the presence of the pathogen, where an alteration in the expression level of the virulence marker in the first subject as compared to the control sample indicates the presence of a pathogen, a pathogen-associated disease, or the virulence of a pathogen. Preferably, the subject is a mammal. More preferred is that the subject is a human. The person of skill will recognize that diagnostic tests may be performed in an array-type format wherein, *e.g.*, the presence of two or more VIRX genes or gene products indicate the presence of a pathogen, a pathogen-associated disease, or the virulence of a pathogen.

VII. ATTENUATED ORGANISMS OF THE PRESENT INVENTION CAN BE USED IN VACCINE PREPARATION

In another embodiment, the invention provides for the use of the attenuated organisms described herein in vaccine preparation. The preparation of vaccines based on attenuated microorganisms is known to those skilled in the art. Vaccine compositions can be formulated with suitable carriers or adjuvants, *e.g.*, alum, as necessary or desired, to provide effective immunization against infection. The preparation of vaccine formulations will be apparent to the artisan. The attenuated microorganisms may be prepared with a mutation that disrupts the expression of any of the VIRX genes identified herein. The artisan will be aware of methods for disrupting expression of particular VIRX genes. Techniques that may be used include, but are not limited to, insertional inactivation, or gene deletion techniques. Attenuated microorganisms according to the invention may also comprise additional mutations in other genes, for example in a second gene identified herein or in a separate gene required for growth of the microorganism, *e.g.*, an *Aro* mutation. Attenuated microorganisms may also be used as carrier systems for the delivery of heterologous antigens, therapeutic proteins or nucleic acids (DNA or RNA). In this embodiment, the attenuated microorganisms are used to deliver a heterologous antigen, protein or nucleic acid to a particular site *in vivo*. Introduction of a heterologous antigen, peptide or nucleic acid into an attenuated microorganism can be carried out by conventional techniques, including the use of recombinant constructs, *e.g.*, vectors, which comprise polynucleotides that express the heterologous antigen or therapeutic protein, and also include suitable promoter sequences. Alternatively, the gene that encodes the heterologous antigen or protein may be incorporated into the genome of the organism and the endogenous promoters used to control expression. In the vaccines of the present invention, the pharmaceutically effective dosage of the mutants of the present invention to be

administered may vary depending on the age, weight and sex of the subject, and the mode of administration. The subject can be, *e.g.*, a human, a non-human primate (such as an ape, gorilla, or chimpanzee), cow, horse, pig, sheep, dog, cat, or rodent (including mouse or rat).

5 VIII. DEFINITIONS

As used herein, each of the following terms has the meaning associated with it in this section.

The term "pathogen," as used herein, is intended to include an agent that causes disease, especially a living microorganism such as a bacterium or fungus. The terms "agent" and "factor" are used interchangeably herein to describe pathogens or toxins useful in the methods of the present invention. Pathogens may include any bacteria, mycobacteria, fungi and unicellular eukaryotic organism, including wild types and mutants thereof, which causes disease or brings about damage or harm to a host organism. Pathogens may also be a poisonous substance, *e.g.*, toxin, which is produced by living cells or organisms and is capable of causing disease when introduced to a host.

The term, "pathogenic," as used herein, is defined as an agent's ability to cause disease, damage or harm to a host organism.

The term, "attenuated," as used herein, means an organism made less virulent relative to an isogenic pathogenic organism.

The term, "virulence," as used herein, is a measure of the degree of pathogenicity of an agent to a host organism. Virulence is usually expressed as the dose of an agent or cell number of a pathogen that will elicit a pathological response in the host organism within a given time period. "Reducing the virulence" as used herein is defined as the ability of a compound to attenuate, diminish, decrease, suppress, or arrest the development of, or the progression of disease, damage or harm to a host organism mediated by a pathogen.

The term, "host organism," as used herein, is intended to include any living organism. Preferably the host organism is a eukaryote, *e.g.*, vertebrate. More preferably the host organism is a mammal. It is most preferred that the host organism be a human.

The term, "mutant," as used herein, an organism carrying a specific mutation of a gene that is expressed in the organism's phenotype.

The term, "mutation," as used herein, is an alteration of one or more nucleic acids of a polynucleotide sequence encoding a gene. A mutation may include the insertion of additional nucleic acids to a polynucleotide sequence encoding a gene, e.g., insertional inactivation of a gene. Alternatively, a mutation may include, but is not limited to, deletion of one or more
5 nucleic acids of a polynucleotide sequence encoding a gene.

The term, "operon," as used herein, is a unit of bacterial gene expression and regulation comprising several genes usually with complementary functions. Typically an operon includes nucleic acid and control elements in the nucleic acid that may be recognized by regulators of gene products. Insertion in a gene in an operon interferes with the function
10 of this gene and of other genes located downstream or upstream in the operon. It is understood herein that the function attributed to a gene refers to its function and/or that of any gene located downstream or upstream in the same operon.

The term, "pharmaceutically effective dosage," as used herein, means that amount necessary at least partly to attain the desired effect, or to delay the onset of, inhibit the
15 progression of, or halt altogether, the onset or progression of the particular condition being treated.

The terms "similarity" and "identity" are known in the art. The use of the term "identity" refers to a sequence comparison based on identical matches between correspondingly identical positions in the sequences being compared. The term "similarity"
20 refers to a comparison between amino acid sequences, and takes into account not only identical amino acids in corresponding positions, but also functionally similar amino acids in corresponding positions. Thus similarity between polypeptide sequences indicates functional similarity, in addition to sequence similarity.

EQUIVALENTS

25 From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that bacterial genes have been identified and assigned a new role in virulence. Further, these genes and their products are useful in the identification of antimicrobial agents, the diagnosis of pathogen-associated disease or infection as well as the preparation of vaccines. Although particular embodiments have been disclosed herein in
30 detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims that follow. In particular, it is contemplated by the inventor that various substitutions, alterations, and

modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. For instance, the choice of the particular pathogen, or combination of pathogens selected for assay or vaccination, the test conditions used in diagnostic assays utilizing the pathogens of this invention, or the method of mutagenesis used to derive the attenuated mutants is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein.

EXAMPLES

This Example is provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Example, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided.

EXAMPLE 1 STRAINS AND CULTURE CONDITIONS USED TO SCREEN FOR ATTENUATED VIURLENCE IN TEST BACTERIAL MUTANTS.

The *D. discoideum* wild-type strain DH1-10 used in these studies is a subclone of DH1 (Cornillon *et al.*, J. Biol. Chem., 275(44):34287-92, 2000). Cells were grown at 21°C in HL5 medium (14.3 g/l peptone (Oxoid), 7.15 g/l yeast extract, 18g/l maltose, 0.64 g/l Na₂HPO₄·2H₂O, 0.49 g/l KH₂PO₄, pH 6.7) (Cornillon *et al.*, J. Cell. Sci., 107 (Pt 10):2691-704, 1994) and subcultured twice a week.

Bacteria were grown overnight at 37°C on Luria-Bertani (LB) agar. Single colonies were inoculated into 5 ml PB (2% (wt/vol) peptone, 0.3% (wt/vol) MgCl₂·6H₂O, 1% (wt/vol) K₂SO₄) (Essar *et al.*, J. Bacteriol., 172(2):884-900,1990) in a 50 ml flask and grown at 37°C for 8 hr prior to use. The growth of various strains was tested in rich medium (PB) by measuring the optical density (600 nm) of a culture at different times after inoculation and was found to be comparable for all strains used. Under these conditions, similar OD_{600s} were obtained for each strain and the induction of quorum sensing was maximal. Minimal Inhibitory Concentrations (MICs) were determined in Mueller-Hinton broth by the microdilution method (Thornsberry *et al.*, NCCLS, 3: 48-56, 1983). Mutations yielding reduced virulence were identified where the growth of the *Dictyostelium* test host organism exposed to the mutant pathogen was greater than the *Dictyostelium* test host organism exposed to wild-type pathogen. Specific genetic mutations in pathogens displaying reduced virulence were identified and characterized by techniques well know in the art.